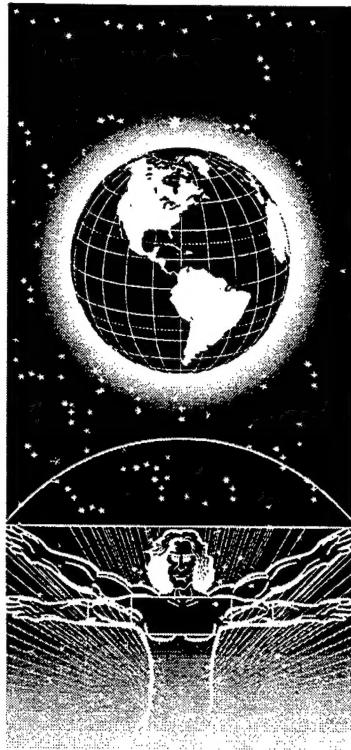


UNITED STATES AIR FORCE  
RESEARCH LABORATORY

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**EFFECTS OF FLIGHT NOISE FROM JET  
AIRCRAFT AND SONIC BOOMS ON  
HEARING, BEHAVIOR, HEART RATE,  
AND OXYGEN CONSUMPTION OF  
DESERT TORTOISES (GOPHERUS  
AGASSIZII)**

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**May 1999**

Final Report for the Period 1 May 1994 to 30 November 1995

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19990831 101

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## TECHNICAL REVIEW AND APPROVAL

AFRL-HE-WP-TR-1999-0170

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public.

This technical report has been reviewed and is approved for publication.

## FOR THE COMMANDER



JOHN F. KENT, COL, USAF, BSC  
Deputy Chief, Crew System Interface Division  
Air Force Research Laboratory

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED	
	May 1999	Final Report - May 1994 - November 1995	
4. TITLE AND SUBTITLE			5. FUNDING NUMBERS
Effects of Flight Noise from Jet Aircraft and Sonic Booms on Hearing, Behavior, Heart Rate, and Oxygen Consumption of Desert Tortoises (Gopherus Agassizii)			C: F336145-89-D-4003 PE: 62202F PA: 7757 TR: C2 WU: 01
6. AUTHOR(S)			
Ann E. Bowles, Ph.D.; Scott Eckert, Ph.D.; Lisa Starke; Erik Berg, Larry Wolski, and Joseph Matesic, Jr.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)			8. PERFORMING ORGANIZATION
Sea World Research Institute Hubbs Marine Research Center San Diego CA			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSORING/MONITORING
Air Force Research Laboratory Human Effectiveness Directorate Crew System Interface Division Aural Displays and Bioacoustics Branch Air Force Materiel Command Wright-Patterson AFB OH 45433-7022			AFRL-HE-WP-TR-1999-0170
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION/AVAILABILITY STATEMENT		12b. DISTRIBUTION CODE	
Approved for public release; distribution is unlimited.			
13. ABSTRACT (Maximum 200 words)			
<p>The effects of simulated subsonic (94.6-114.2 dB CSEL) and supersonic (0.25-6 psf) aircraft noise on 14 desert tortoises, <i>Gopherus Agassizii</i> were studied. Auditory thresholds of the most sensitive individuals at 250 Hz were 17.5 dB SPL. Thresholds averaged 34 dB SPL at 250 Hz for the 11 individuals tested. No significant temporary threshold shift (TTS) was detected after worst-case exposure to subsonic aircraft noise or 2-6 psf simulated booms. TTS ranging from 5-20 dB was measured in 5 of 9 individuals after exposure to 10-6 psf booms (recovery &gt; 45 min and &lt; 48 hr). Tortoises were trained to walk voluntarily on a treadmill and fitted with small mask for measurements of O<sub>2</sub> consumption. VO<sub>2</sub> at rest averaged 1.472 ml/min/kg (s.d.=0.410). Resting heart rate averaged 11.82 BPM (s.d. = 3.91). Exercising heart rate ranged from 10 BPM to 42 BPM, averaging 30 BPM. There was no increase in blood lactate levels during or post-exercise. The most extreme response to simulated subsonic aircraft noise was a typical reptilian defensive response - freezing - for up to 113 minutes. After the initial exposure, bouts were short (&lt;~5 min); thereafter, the tortoise entered a milder defensive state (head withdrawals, alerting, less climbing or digging). Averaged heart rate declined 7-8% decrease during exposures, corresponding to the shift in behavior. Responses to sonic booms were limited to brief bouts of alerting.</p>			
14. SUBJECT TERMS			15. NUMBER OF PAGES 155
Aircraft, Aircraft Noise, Desert Tortoise, Effects, Overflight, Habituation, Behavior, Disturbance, Sonic Booms, Heartrate			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED

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## FOREWORD

This study was prepared by personnel from the Hubbs-Sea World Research Institute (HSWRI) under the direction of Ann E. Bowles, Ph.D. and Scott Eckert, Ph.D., co-principal investigators. The effort was funded under contract F33615-89-D-4003, Order 0132, to Parsons Engineering Science, under the direction of Dr. R.C. Wooten, Jr. The program is part of the F-22 development effort, and is in support of a Section 10 consultation between the U.S. Fish and Wildlife Service, Division of Consultation and Conservation Planning, Portland Regional Office, and the Environmental Management Office, Air Force Flight Test Center, Edwards Air Force Base. The experiments were allowed under U.S. Fish and Wildlife Service Regional Blanket Permit PRT 702631, Subpermit ED-AFB, "Recovery subpermit to take the desert tortoise (*Gopherus agassizii*)", which expires June 30, 1996. Mark Hagan at Edwards Air Force Base was responsible for capture, management, and final disposition of the tortoises. This contract was managed by Lt. Col. Robert Kull, Armstrong Laboratory, Occupational and Environmental Health Directorate, Brooks Air Force Base, and Captain Michael Carter, Armstrong Laboratory, Noise Effects Branch, Wright-Patterson Air Force Base.



## ACKNOWLEDGMENTS

We gratefully acknowledge the assistance and advice of a large number of individuals in the performance of this contract. Wanda Deal gave up quality weekend time to capture the tortoises and deliver them safely to San Diego, and later to return them to Edwards Air Force Base. The San Diego Turtle and Tortoise Society, San Diego Zoological Society, and Al Myrick provided invaluable advice on desert tortoise husbandry. Sea World of California provided many hours of assistance without charge, particularly the staff of the Animal Care, Security, Electronic Services, and Special Services departments. Special kudos go to Craig Hill in Special Services for building a cage assembly for the playback experiments, to Greg Cain for providing a low frequency speaker, and to Jim McBain and Tom Reidarson for providing veterinary care in emergencies, taking x-rays, running blood tests, and giving medical advice at odd hours without complaint. Jane Meier, Pamela Yochem, and Jeff Jenkins provided expert veterinary care. Joseph Matesic, Jr., did a fine job of designing and building the Impulse Noise Test Facility. Annette Henry acclimated the tortoises to captivity with plenty of patient handfeeding; she and Tami Mau provided the tender, loving care that allowed the tortoises to increase weight by an average of 20% over the course of the experiments. Zack Dembitsky volunteered his services as a physiological technician. Jon Francine was responsible for setting up the initial phases of the hearing sensitivity and noise exposure experiments. Scott Yaeger worked many patient hours to collect auditory measurements in a room that was a bit too warm. Scott, Ian Gini, and Scott Biggers struggled to build habitats and move dehumidifiers around until conditions were warm enough (but not too warm), dry enough, and quiet enough. Paul Ponganis, Tobias Wang, Jim Hicks, Darlene Ketten, and many other colleagues provided helpful advice. The Center for Marine Biotechnology and Biomedicine and Hydraulics Laboratories at Scripps Institution of Oceanography provided equipment for metabolic rate measurements; special thanks go to Gerald Kooyman and Markus Horning, without whom the physiological measurements would not have been possible. Bio-logic Systems Corp. has been a loyal supporter of the Hubbs-Sea World Research Institute for many years—they donated the audiometric system used for the hearing experiments.



## TERMS AND ABBREVIATIONS

Unless otherwise specified, all acoustical terms are drawn from Harris (1991). A glossary of these terms is given in Appendix A.

<b>ABR</b>	auditory-evoked brainstem response
<b>A/D</b>	analog-to-digital converter
<b>AEP</b>	auditory-evoked potential
<b>AFFTC</b>	Air Force Flight Test Center, Edwards Air Force Base
<b>ANSS</b>	U.S. Air Force Aircraft Noise Simulation System
<b>ASEL</b>	A-weighted sound exposure level
<b>BPM</b>	heart beats per minute
<b>CSEL</b>	C-weighted sound exposure level
<b>cm</b>	centimeter (metric abbreviation)
<b>D/A</b>	digital-to-analog converter
<b>DAT</b>	digital audio tape
<b>EAFB</b>	Edwards Air Force Base
<b>ECG</b>	electrocardiogram
<b>EFR</b>	envelope-following response
<b>FFR</b>	frequency-following response
<b>FFT</b>	fast Fourier transform
<b>ha</b>	<i>hectare</i> (metric abbreviation)
<b>HSWRI</b>	Hubbs-Sea World Research Institute
<b>Hz</b>	Hertz, cycles per second
<b>IACUC</b>	Institutional Animal Care and Use Committee
<b>INTF</b>	HSWRI Impulse Noise Test Facility
<b>L<sub>max</sub></b>	maximum, fast, A-weighted sound pressure level
<b>ms</b>	milliseconds (metric abbreviation)

**overpressure** peak pressure of an impulse minus ambient pressure

<b>Pa</b>	Pascals
<b>peak</b>	largest absolute value of sound pressure
<b>psf</b>	per square foot
<b>s</b>	seconds (metric abbreviation)
<b>SEL</b>	sound exposure level
<b>SNR</b>	signal-to-noise ratio
<b>sound level</b>	fast, A-weighted sound pressure level.
<b>SPL</b>	sound pressure level.
<b>STP</b>	standard temperature and pressure
<b>STPD</b>	standard temperature and pressure dry
<b>TTS</b>	temporary threshold shift
<b>PTS</b>	permanent threshold shift
<b>URTD</b>	upper respiratory tract disease
<b>USAF</b>	U.S. Air Force
<b>VibBR</b>	vibration-evoked brainstem response

## SUMMARY

Testudinates (turtles and tortoises) have been characterized as deaf because many species exhibit little response to sounds in their environment. However, they possess auditory organs, and the few species that have been examined can hear, even if not with great sensitivity. Several species of tortoises, including the threatened desert tortoise (*Gopherus agassizii*), have acoustic social signals, and are known to react to sounds produced by predators. Therefore, there is concern that high-intensity subsonic aircraft noise and sonic booms could stimulate potentially-damaging responses in the desert tortoise or affect their ability to perceive biologically-meaningful sounds.

The reactions of turtles and tortoises to high intensity transient noise have not been studied previously. Species typical defensive responses of testudinates to the approach of danger include startling, running, diving (marine species), wedging the shell into a crevice, urinating and defecating on an attacker, producing threatening sounds, freezing, and withdrawing into the shell. Based on discussions with project investigators and the U.S. Fish and Wildlife Service, the plausible potentially-damaging effects of noise from aircraft were water loss if a water-deprived animal was frightened into urinating; long-term changes in normal activity patterns; increased energetic expenditures resulting from increases in heart rate or activity; inappropriate behavioral responses (e.g., emerging from the burrow in the heat of summer); noise masking of biologically-important sounds; and damage to hearing. The Desert Tortoise Recovery Plan (Desert Tortoise Recovery Team 1993) specifically listed masking of social signals or other important natural signals and hearing damage as potential impacts of jet aircraft noise.

The U.S. Air Force (USAF) Aeronautical Systems Center will test the F-22 on Air Force Flight Test Center (AFFTC) ranges at Edwards Air Force Base (EAFB), California. Hubbs-Sea World Research Institute was contracted to conduct an experimental study to determine whether any of the potential effects from F-22 noise posed a significant risk to desert tortoises. The experiments reported herein were designed to obtain a first-order estimate of effects on hearing, behavior, and energy metabolism.

The F-22 is expected to operate from 5,000-50,000 ft AGL at speeds of 0.3-2.0 M. Training exercises will produce carpet booms (N-waves) in straight-and-level flight and focused booms (complex waveforms) during acceleration and maneuvering. Carpet booms were projected to reach 8 psf in the worst case, with a duration of ~100 ms and fastest-projected rise time of 0.4 ms. Focused booms were projected to reach a worst-case peak level of 25 psf. Training exercises will also produce subsonic aircraft noise, with estimated worst-case level of 110 dB ASEL for indi-

vidual overflights and a maximum of 45 min of intermittent exposure at any one location.

The goals of the present study were as follows: (1) to measure the auditory sensitivity of desert tortoises and determine the influence of vibration sensitivity thereon; (2) to determine whether tortoises suffered temporary loss of hearing after exposure to simulated subsonic and supersonic aircraft noise; (3) to measure behavioral and cardiac responses to aircraft noise; (4) to measure the relationship between heart rate and metabolic rate; and (4) to use this relationship to estimate changes in energy consumption after noise exposure.

Fourteen desert tortoises were captured at the site of a planned development project in Barstow, California in late February of 1995 and transported to HSWRI, where a habitat was prepared to hold them. The tortoises were held within the estimated preferred temperature and humidity range of desert tortoises (28-32°C, 18-30% humidity) and under a summer day-light cycle (16 hr day-light, 8 hr darkness). Tortoise weights ranged from 1.2 to 3.6 kg at capture (carapace lengths 19.7-31.4 cm). One individual had mild symptoms of upper respiratory tract disease (URTD) at capture, which were treated; in addition, tortoises were treated for intestinal parasites and anemia. They were maintained in a hydrated condition with fresh food daily in order to counteract the potential stresses of captivity. Each tortoise was individually housed to prevent the transmission of disease and to eliminate social interactions that would complicate the interpretation of behavioral observations. During the course of the study, tortoises increased weight by an average of 20%.

Auditory thresholds were measured using auditory evoked potentials in a sound isolation chamber with the external temperature held within the 28-32°C range. At these temperatures, the most sensitive individuals had best sensitivities of approximately 20 dB SPL, while the least sensitive had thresholds in excess of 50 dB SPL. Thresholds averaged 34 dB SPL at best frequency, making the tortoises approximately 10 dB more sensitive than expected. Hearing sensitivity declined rapidly above 750 Hz and below 125 Hz.

Collection of auditory thresholds using far-field (non-invasive) electrophysiological methods is always difficult at frequencies below ~250 Hz. However, tortoises were expected to hear down to approximately 50 Hz. Therefore, from the start of the study, it was expected that measurements made at the low-frequency end of the tortoises' hearing range would be difficult. In addition, it was expected that sensitivity to vibration would become the dominant sensory modality at very low frequencies (below ~100 Hz). Therefore, several methods were tested in an effort to improve the accuracy of measures of low frequency sensitivity - otoacoustic emissions, vibration-evoked

potentials, and the modulation-rate transfer function. Unfortunately, none proved useful. At low frequencies, even low-amplitude crosstalk between the input and output modules of the evoked-potential measurement system made electrophysiological signals uninterpretable. Also, the signal to noise ratio of otoacoustic emissions from the desert tortoise ear were too low to permit accurate estimates of threshold.

An IAC Sound Isolation Chamber was equipped with a USAF Aircraft Noise Simulator designed to project intense subsonic aircraft noise. Measurements of auditory sensitivity were collected from 6 tortoises before and after exposure to 20 subsonic aircraft overflights with levels from 94.6 to 114.2 dB CSEL, totaling 40 minutes of exposure. Peak levels of these overflights reached 126.1 dB. No significant temporary threshold shift (TTS) was detected even in the most sensitive individuals after this worst-case exposure.

A sonic boom with an 0.4 ms rise time contains substantial energy in the range from 125 to 1000 Hz, the range within which auditory injury in desert tortoises could occur. Therefore, all simulated sonic boom exposures had this rise time, despite the fact that typical carpet and focused sonic booms have rise times in the range from 1-10 ms. The highest peak overpressure that could be generated in the HSWRI Impulse Noise Test Facility (INTF) with a rise time of 0.4 ms was 6 psf. This level was within 3 dB of the highest level anticipated from a carpet boom from the F-22 (8 psf or 143 vs. 146 dB flat-weighted peak SPL). In order to guarantee a worst-case exposure, two 6 psf booms were presented in quick succession. The hearing of 5 tortoises was tested before and after exposure to these sonic booms. No significant (detectable) TTS was detected.

Tortoises were also exposed to cumulative energy equivalent to that of a 25 psf sonic boom by presenting them with 10 simulated sonic booms. It is recognized that this method was likely to underestimate non-linear responses resulting from high peak level exposure and overestimate temporary hearing loss due to repeated exposure, but design limitations of the INTF prevented presentation of 25 psf booms with fast rise times. TTS ranging from 5-20 dB was measured in 5 of 9 of the tortoises tested; recovery times were usually < 1 hr, but one individual recovered > 1 hr and < 48 hr. The small shift and rapid recovery suggested that desert tortoises can tolerate occasional exposures at this level without injury. However, the shift does suggest that a proportion of desert tortoises could develop hearing deficits as a result of frequent exposure to focused sonic booms and other high-amplitude impulses over a lifetime (e.g., off-road vehicle noise, blasts from construction). Also, because the peak level of the test booms was close to the damage risk threshold adopted for humans and animals (140 dB peak SPL), the critical level for damage to tortoises may not be higher than that for humans and laboratory animals, as had been expected as a result

of their less-sensitive hearing.

Tortoises were also exposed to subsonic aircraft noise and simulated sonic booms during behavioral monitoring experiments. Half the tortoises exposed using this protocol were equipped with heart-rate monitoring leads to determine cardiac responses and metabolic effects. Results of both series of experiments showed that, while the tortoises exhibit startle responses (muscular flinching, increases in heart rate, abrupt movements) after being touched, they did not have an acoustic startle response; no muscular flinch was observed and no abrupt change in heart rate could be measured.

Instead, exposures to simulated jet overflights produced another typical reptilian defensive response, freezing. The behavioral change was abrupt during initial exposures. During or shortly after exposure onset, tortoises frequently became immobile for periods of up to 113 minutes, interrupting activities such as walking or eating. Freezing was abrupt, with the head and appendages often left extended. This behavior occurred in 30% of tortoises (N= 12) during the initial day of exposures. It did not always occur at the start of the first exposure. Freezing habituated rapidly. In subsequent exposures on another day, no tortoises froze for an extended period.

When tortoises did not freeze, other common reptilian defensive responses were observed. Tortoises looked overhead as though localizing the sound source, then remained somewhat more defensive and vigilant during the experiment. Head withdrawals were observed during exposures in 11 of 12 experiments, as opposed to one of 12 during baseline observations. The vigilant behavior of the tortoises resulted in a reduction of energetic activities (pacing; climbing). As a result, averaged heart rate in the hour after exposure showed a 7-8% decrease relative to the hour before. This vigilance did not habituate detectably across the three exposures delivered on a single day nor the first and second day of exposures in the few cases where the experiment was repeated (N= 3 tortoises).

Behaviors of tortoises were also observed during exposures to simulated sonic booms. They received (1) two series of ten sonic booms at levels from 0.25 to 4 psf, and (2) intermittent exposures to 4 single sonic booms ranging from 1 to 6 psf. Tortoises did not freeze after initial exposures to any sonic booms. Typically they looked around briefly, then returned to their previous activity. Head withdrawals were not observed. Both increases and decreases in activity followed exposures and the changes could not be linked causally to simulated booms. There was no relationship between heart rate or changes in activity and the level of the boom. Orienting responses disappeared with successive exposures, suggestive of habituation.

Measurements of heart rate, metabolic rate, and blood lactate levels were collected from resting and exercising tortoises. Resting heart rates of eight tortoises ranged from 6.15 to 18.03 beats per minute (BPM), with a mean of 11.82 BPM ( $sd=3.91$ ). Tortoises walked readily on a treadmill when provided with food or an escape route as motivating stimuli, as long as the treadmill remained within their preferred speed range (0.02-0.12 m/s). Tortoises could be stimulated to walk within the preferred speed range for periods in excess of an hour. Exercising heart rates ranged from 10 to 42 BPM, typically from 20 to 35 BPM.

Metabolic rates were measured in 8 tortoises while resting and exercising. Five of these tortoises were implanted with a jugular catheter to allow blood sampling during exercise, so that blood lactate levels could be measured. Resting metabolic rates ranged from  $0.913-0.996 \text{ ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$  (mean= 1.472,  $sd=0.410$ ). Resting blood lactate levels ranged from 0.1 to 0.6 mmol/l. While exercising, metabolic rates ranged from  $\sim 2-7 \text{ ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$ , but blood lactate levels did not increase detectably, indicating that the exercise was within the aerobic scope of the tortoises.

Heart rates and metabolic rates were correlated by fitting the data to an exponential function. The correlation was high ( $r=0.74$ ) with residuals uniform and small throughout the measurement range. The best-fit functions differed somewhat among individuals, but the differences were not large. Therefore, the exponential model developed from these data could be used to predict metabolic rate of unknown individuals if heart rates were collected.

Tortoises were stimulated to exceed their aerobic scope by handling. During this exercise, blood lactate levels peaked at 2.7 mmol/l after 22 minutes of exercise at a sustained heart rate of 41 BPM. Such intense exercise may occur when tortoises climb persistently, during fights, or during attempts to right themselves after a fall. Aside from such intense periods of exercise, the excellent correlation between heart rate and metabolic rate will make it possible to estimate the cost of increased activity after exposure to aircraft noise during active periods (summertime). The relationship between temperature, heart rate and metabolic rate was not measured. Therefore, the results of this study must be extrapolated with caution outside the range from 28-34 °C.

The tortoises frequently urinated and defecated on days when they ate well and rarely when they did not eat. Defecation and urination were not expected during noise exposures after it was determined that the tortoises did not have an acoustic startle, but it was possible that changes in activity could have stimulated changes in voiding rates. However, the rate of urination and defecation *relative to food consumption* was not detectably different during exposure experiments. Because the tortoises ate less in the experimental chambers, voiding rates were actually lower

under exposure conditions.

The measurements made during these experiments were conducted at temperatures typical for tortoises during active periods in the summertime (28-34°C). Small changes in temperature produced large changes in auditory sensitivity and activity, however, suggesting that the results of these experiments cannot be extrapolated to low temperature conditions.

The results reported herein are best viewed as a first-order effort to determine the effects of sub-sonic and supersonic aircraft noise on a desert reptile. They provide evidence that hearing loss and physiological changes are not likely to be dangerous during occasional short-term exposures to intense transients. The results cannot be extrapolated directly to chronic exposures over a tortoise's lifetime. The extent of tortoise habituation under natural conditions cannot be predicted, for example. Also, because tortoises experienced TTS after worst-case exposures, it is possible that permanent effects on hearing would be observed if tortoises were exposed to focused booms often (weekly to daily over many years).

Several lines of inquiry could be pursued profitably if further information on the impact of noise is required. Changes in activity with repeated exposure to aircraft noise should be investigated under natural conditions, including during food and water deprivation, torpor, or exposure to dangers such as rivals and predators. In addition, if areas of frequent sonic boom exposure are found in aircraft operations areas, damage risk criteria for tortoise hearing should be established. The effect of temperature on auditory sensitivity and the heart rate-metabolic rate relationship should also be quantified to make predictions possible under all conditions.

## INTRODUCTION

Testudinates (turtles and tortoises) have been characterized as deaf because many species exhibit little response to sounds in their environment. However, they possess auditory organs, and several species of tortoises, including the threatened desert tortoise (*Gopherus agassizii*), produce acoustic social signals and are known to react to meaningful sounds in their environment, including sounds produced by predators. Therefore, there is concern that high-intensity subsonic aircraft noise and sonic booms could stimulate potentially-damaging responses or affect their ability to perceive meaningful sounds. Hubbs-Sea World Research Institute (HSWRI) was contracted to conduct an environmental study on the effects of F-22 subsonic and supersonic aircraft noise on desert tortoise behavior, hearing and energy metabolism.

The U.S. Air Force (USAF) Aeronautical Systems Center will test the F-22 on Air Force Flight Test Center (AFFTC) ranges at Edwards Air Force Base (EAFB), California. The F-22 will be operated from 5,000-50,000 ft AGL at speeds of 0.3-2.0 M. Based on previous experience with other fighter aircraft and sonic boom prediction models, training exercises will produce carpet booms (N-waves) in straight-and-level flight and focused booms (complex waveforms) during acceleration and maneuvering. Worst-case carpet booms were estimated to reach 8 psf with a worst-case rise time of 0.4 ms and duration of ~100 ms. Worst-case focused booms were estimated to reach a peak level of 25 psf. Training exercises will also produce subsonic aircraft noise, with estimated worst-case level of 110 dB ASEL for individual overflights and a maximum of 45 min of intermittent exposure at any one location.

Almost nothing is known about the behavioral and non-auditory physiological responses of reptiles to sound. Species typical defensive responses to the approach of danger include alerting, startling, running, diving, urinating, freezing, and withdrawing into the shell. It is not known what part of this repertoire testudinates exhibit to intense sounds, although a handful of anecdotal accounts indicate that freezing is a common reptilian response (Wever and Vernon 1960). Based on this evidence, injury from panics is highly unlikely. Instead, the plausible potentially-damaging responses are (1) water loss when a water-deprived animal urinates, (2) long-term changes in normal activity patterns, (3) increased energetic expenditures resulting from increases in heart rate or activity, (4) inappropriate behavioral responses (e.g., emerging from the burrow in the heat of summer), and (5) hearing damage, although the latter is only likely at very high sound exposures. The desert tortoise recovery plan (Desert Tortoise Recovery Team 1993) lists masking

of biologically-important signals as well, but sonic booms are of such short duration that they are unlikely to mask any signals significantly. Subsonic noise that exceeded typical ambient noise levels in desert tortoise habitat could mask biologically-important sounds (e.g., predators approaching); however, the worst case exposures predicted for the F-22 would result in a duty cycle for masking noise of less than 2% (that is, tortoises would spend less than 2% of their time in the presence of noise sufficient to mask biologically-significant sounds).

The literature pertinent to each potential effect is reviewed below. It may as well be said in advance that none of it provides evidence that aircraft noise would be either harmful or harmless to any reptile, including the desert tortoise. In the face of this absence of data, environmental impact from F-22 activities could not be predicted. After discussion with U.S. Fish and Wildlife Service managers and project scientific staff, the goals of the present study were established as follows: (1) to measure the auditory and vibration sensitivity of desert tortoises; (2) to determine whether tortoises suffered temporary loss of hearing after exposure to simulated subsonic and supersonic aircraft noise; (3) to measure behavioral and cardiac responses to noise exposures; (4) to measure the relationship between heart rate and metabolic rate; and (4) to use this relationship to estimate changes in energy consumption after noise exposure. Because so little is known about auditory, behavioral, and metabolic effects of disturbance on any reptile, this research was regarded as a first order investigation, determining whether any of the potential effects merited further investigation and testing techniques that could be used to measure effects directly in the field.

#### **Natural History and Behavior of the Desert Tortoise**

Desert tortoises belong to the family *Testudinidae*, comprising 10 genera and 32 species worldwide. One genus, *Gopherus* (gopher tortoises), is native to the United States (U.S.), with three species (the desert tortoise, gopher tortoise [*G. polyphemus*], and Berlandier's tortoise [*G. berlandieri*]). All species are strictly terrestrial. They are protected by a heavy carapace and plastron joined by a well-developed bridge, and have thick, hard scales on the legs and head. All three species are herbivorous, living in warm desert or scrublands of the southern U.S. They feed on grasses, forbs, succulents, trees and shrubs.

The desert tortoise lives in arid sandy or gravelly desert areas of eastern California, southern Nevada and Utah, western Arizona, and northern Mexico, areas dominated by creosote-bursage and Sonoran desertscrub plant communities. The Mojave Desert population is listed as threatened.

The desert tortoise can reach 37 cm in carapace length and weigh up to 5 kg. Estimates of their

maximum longevity are in excess of 100 years, with estimates of age at first reproduction ranging around 15-20 years. Populations from California and Nevada are considered distinct and are managed separately. The desert tortoises used in this study were from the vicinity of Barstow, in the lower Mojave Desert of California.

Desert tortoises are fossorial (burrow-dwelling). Their front legs are flattened and heavily armored, and they have strong claws and large flat feet on all four legs. These adaptations make them good climbers and diggers; the armored legs also provide a protective barrier when pulled across the opening of the shell or burrow. They can extend their legs backward or sideways above the plane of the carapace and rotate with considerable force, an adaptation for righting themselves after being upset in a fall or fight. They dig their own burrows or inhabit burrows of other species; burrows can measure up to 10 m in length, but are usually under 5 m long and 1-1.5 m deep.

Temperature, the annual day-night cycle, rainfall, and availability of fodder probably entrain the reproductive cycle. During cool winter weather, tortoises hibernate, emerging under the stimulus of warming weather and spring rains to mate. They nest during the late spring and early summer. In the Mojave (Rostal *et al.* 1994), they also mate in the fall before hibernation begins. Nesting in the Mojave takes place from May-early July, with females laying 2-3 clutches of 2-7 eggs. The eggs are deposited in funnel-shaped nests 15 cm deep, sometimes located in the mouths of their burrows. The young hatch from mid-August to October, when summer monsoon rains stimulate a late summer period of growth and productivity.

Like most desert vertebrates, their circadian rhythms are linked to temperature and the day-night cycle. During the warm months, they emerge from their burrows during early morning and evening hours for several hours every few days while food and water are available. They estivate during mid and late summer after annual plants and grasses dry up, re-emerging during the late summer monsoons. In other desert species, emergences are stimulated by rain and the sound or ground-induced vibrations of thunderclaps (Dimmitt and Ruibal 1980), but the relevant stimuli are not known for the desert tortoise. In the fall and early spring, they emerge to forage during the noontime hours, when conditions are warm enough for activity.

O'Connor *et al.* (1994b) studied movement patterns of the desert tortoise in the eastern Mojave Desert. Tortoises had home ranges in the sense that they moved within a circumscribed area, but the area shifted over seasons, and the tortoises did not appear to exclude conspecifics. Home ranges varied from 5.9-46.0 ha, with males covering larger areas than females. The home ranges

were so large that the tortoises could not revisit much of them during the course of an active period, and there was little overlap between areas used during the spring vs. late summer. O'Connor *et al.* (1994b) concluded that the home range estimates they obtained were useful for estimating the extent of tortoise movements, but that they could not be considered evidence of any 'home' area or territory. Males moved greater distances than females, regardless of season. In a study by Goldsmith and Shaw (1990), home ranges within active periods had an average greatest length of ~500 m (range 160-985 m). In a study of the Sonoran population in the Picacho Mountains of Arizona, Barrett (1990) found average home range sizes of  $19.07 \pm 4.63$  ha (range 3-53 ha), and Schwartzmann (1983) found average home range sizes of  $20 \pm 8$  ha (males) and  $13 \pm 4$  ha (females), consistent with the results of O'Connor *et al.*

Ruby and Niblick (1994) developed an ethogram (enumeration of behavioral repertoire) for the desert tortoise. They describe its defensive behaviors, in rough order of intensity: scanning the environment (=looking around), freezing, lying down flat (usually preparatory to withdrawing into the shell), withdrawing into the shell (head only or head and legs), backing into the burrow, wedging the shell into a confined space, running away and voiding water. Of these, freezing and lying down were the most common responses. Lying down could occur slowly or abruptly, depending on the tortoise's perception of risk. Running generally occurred when one tortoise escaped attack by another tortoise, but Ruby and Niblick also observed the behavior in response to sprinklers in an irrigation system.

Little is known about the predators of desert tortoises, and most of the evidence available treats the Mojave population. At present, the most important predators are ravens and coyotes (Desert Tortoise Recovery Team 1993), although eggs, hatchlings, and juveniles are taken by numerous other predators. Reptile predators include the Gila monster (Barrett and Humphrey 1986), gopher snakes, and coachwhip snakes, but not rattlesnakes (Johnson *et al.* 1990). Hatchlings and juveniles are also taken by birds (ravens, red-tailed hawks, golden eagles) and mammals (bobcats, coyotes, kit foxes, grey foxes, badgers, skunks, ringtail cats, and cougars; Johnson *et al.* 1990). Adult desert tortoises are relatively invulnerable to all but the largest predators. Anecdotal accounts indicate that hatchlings are aggressive when disturbed, hissing and biting (Luchenbach 1982); adults hiss and withdraw into their shells, presenting predators with the heavily armored scutes on their fore and hind limbs.

Ruby and Niblick reported a number of behaviors indicating that the tortoises obtained important information about their environment via the chemosensory system. Tortoises often investigated objects and other tortoises by sniffing or touching with the nose. Alberts *et al.* (1994) showed that

the chin gland could be used for chemical communication. Desert tortoises scent mark the scales on their forelegs and rub the shells of rivals with the chin glands; but they do not rub them on other objects in their environment, consistent with the hypothesis that they defend themselves and their mates, but do not maintain an established territory. Both sexes rub with the chin glands, but the behavior is most common in courting males, which possess the largest chin glands.

Most authors report that tortoises primarily use chemical and visual signals in communication. Many observers have reported that tortoises are deaf because they do not appear to respond to sounds or respond to vocal signals. However, several authors have described a complex acoustic repertoire for the desert tortoise (Patterson 1971, 1976; Campbell and Evans 1967; Ruby and Niblick 1994), which includes hissing, grunting, grinding the mandibles, screeching, and low, prolonged moaning. The frequencies of the sounds range from around 200 Hz to 4.5 kHz, but levels are low in the case of the desert tortoise. The function of these calls is still a matter of controversy, however. Some authors maintain that many of these sounds are unintentional, the accidental result of activity. For example, Ruby and Niblick were of the opinion that sounds made during mating were a by-product of the mating process rather than a form of communication. However, tortoises worldwide are infamous for the loud grunts and moans that occur when they mate, sounds that do not occur during other high activity states, such as in fights. This suggests a communicative function for the behavior. Hisses, screeches, and grinding of the mandibles occur during agonistic encounters.

The courtship and agonistic behavioral repertoire of the desert tortoise is relatively complex; as tortoises in this program were housed singly, this report will not consider such interactions. However, some of the elements of the agonistic repertoire were seen occasionally in isolation, particularly head-bobbing and chin rubbing.

Desert tortoises also exhibit a number of comfort and maintenance movements, including digging and pushing dirt with both front and rear legs; grasping, tearing, mashing, and gulping food; sniffing, nosing, and mouthing objects or other individuals; drinking; yawning; self-righting; and basking. Activity states, in rough order of increasing energetic expense, were: sleeping, sometimes with the head and legs stretched out on the ground; sitting or lying quietly alert with the weight off the legs; standing; slow walking; normal walking; courtship movements; fast walking; running; fighting and righting themselves; and climbing.

Desert tortoises travel long distances to find mates, avoid adverse environmental conditions, and find good areas to hibernate. In both captive and wild environments, they respond to barriers by traveling along them until they find a way to go around (Ruby *et al.* 1994a). Human-made barri-

ers with no openings large enough to permit passage stimulate tortoises to investigate and attempt to escape, often persistently for periods of hours (up to 18 hours in the experimental study by Ruby *et al.*) This response declined with time in laboratory experiments, but did not disappear completely. In the laboratory, tortoises tended to climb in corners more often than along curved surfaces. They preferred to walk along a surface rather than attempt to dig under it.

Desert tortoises drink water from puddles after a rainstorm. They travel to familiar sites to obtain drinking water after storms (Berry 1972), and they may also dig small depressions that serve to collect a supply of drinking water during storms (Medica *et al.* 1980). They emerge immediately after rainstorms, suggesting that they respond to sensory cues associated with the storms. Vibration is likely to play an important role, as tortoises can be stimulated to emerge from their burrows by slapping the ground nearby (Desert Tortoise Recovery Team 1993).

### **Measuring Sensitivity of Reptiles to Sound and Vibration**

Sound reception in turtles and tortoises occurs along a pathway homologous to the familiar mammalian auditory pathway, but with greater limitations in frequency range and absolute sensitivity. In testudinates, sound enters the ear via the skin of the head (analogous to the mammalian tympanic membrane, but with no external ear or auditory canal). In terrestrial turtles and tortoises, there is a tympanum, a thin circle of skin like a drumhead over the ear (Figure 1). Sound passes through it and into the extracolumella, which at the outer end is a flattened plate of bone lying underneath the tympanum. The extracolumella narrows to a slender point, connecting to the columella within the fluid filled middle-ear cavity. This rod extends through the quadrate bone and into the pericapsular recess, where it widens into a footplate (the stapedial footplate) that abuts on the pericapsular membrane that surrounds the otic capsule and the saccule, the organs of hearing and balance, respectively.

In terrestrial most animals, the bones of the middle ear match the impedance of a low-density medium (the air) to a high density medium (the fluids of the inner ear). In testudinates, the matching is poorer, making sound transmission via the specialized structures of the outer and middle ear less efficient, and increasing the importance of sound conduction through other tissues into the inner ear (commonly called bone conduction). This is thought to explain the relative insensitivity of testudinates in air. Underwater, where the impedance mismatch between the external medium and the animal's tissues is small, differences between the two pathways are potentially small. However, experiments with both airborne sound sources and vibrating rods held against the tissue have shown that testudinates achieve their best sensitivity when sound enters via the specialized structures of the auditory pathway (Ridgway *et al.* 1969), and that their sensitivity is considerably

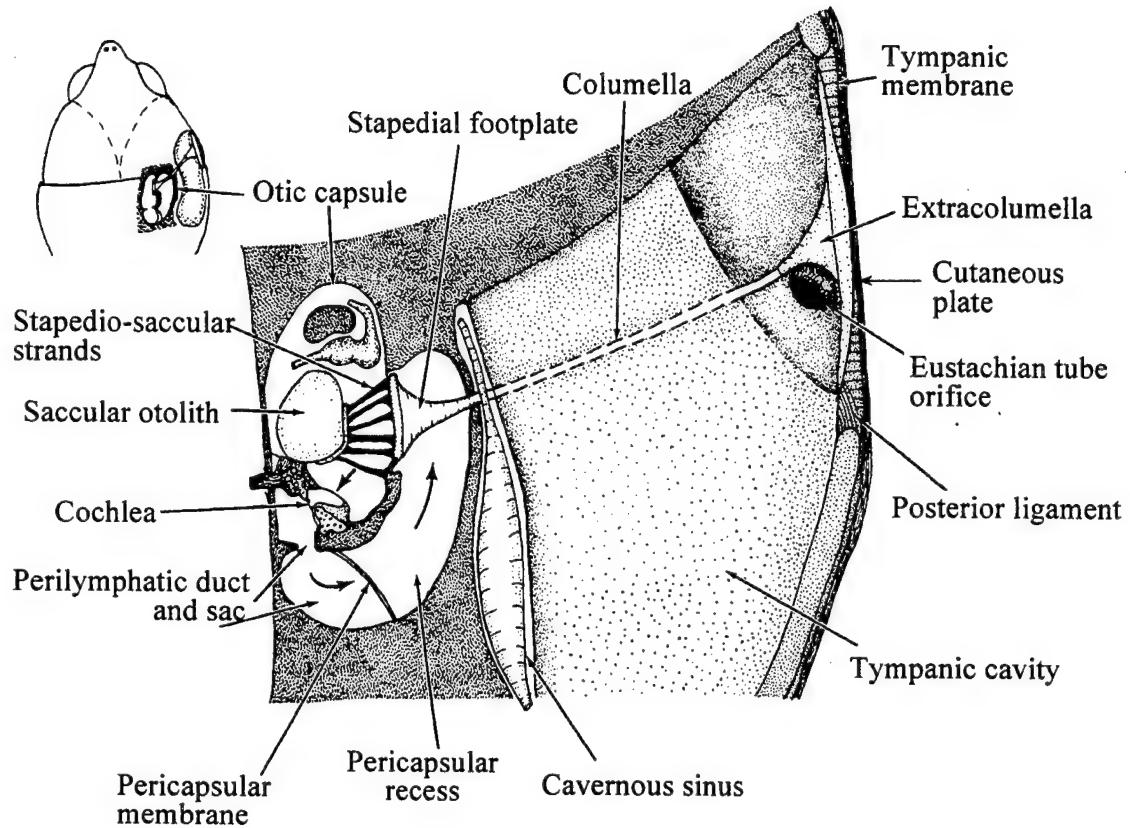


Figure 1. The anatomy of the turtle ear. Arrows indicate the fluid flow produced by an inward thrust of the stapedial footplate (after Wever and Vernon 1956b).

reduced after sectioning the columella (Patterson and Gulick 1966) or removal of the tympanum (Fay 1988).

Studies that measure hearing sensitivity using positive or negative reinforcement techniques (=behavioral measures) are widely regarded as the most sensitive and accurate measures of absolute hearing threshold. Although reptiles are quite capable of learning, especially when the desired responses are species-typical behaviors elicited by food or noxious stimuli, their performance tends to be slow and unreliable, making behavioral tests prohibitively time-consuming (Suboski 1992). Only one study has measured the hearing of any species of testudinate using a behavioral measure, Patterson's study of the pond slider (most likely *Pseudemys* [=*Chrysemys*]

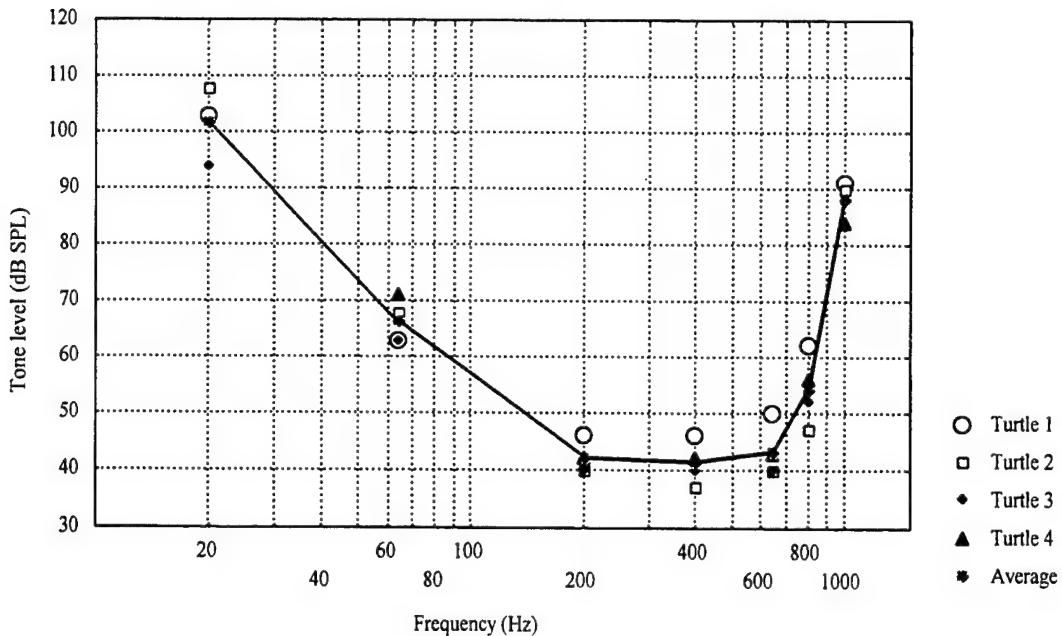


Figure 2. Auditory threshold functions of four *Pseudemys scripta* measured by Patterson (1966) using negative reinforcement.

*scripta*; Patterson 1966, Patterson and Gulick 1966).

Patterson's turtles heard best in the band between 20 and 700 Hz, with best sensitivities of 45-50 dB SPL (Figure 2). Sensitivity declined rapidly above and below. Patterson also measured thresholds for detection of acceleration (vibration) on the carapace. Sensitivity to vibration was fairly constant from 20 to 640 Hz, ranging from -10 to 0 dB re 1 in/s<sup>2</sup>, and rolling off steeply above 640 Hz. Patterson cut the columella and found no decrease in vibration sensitivity, but substantial hearing loss above 200 Hz. Patterson noted that after transection of the columella the turtles responded to airborne sound only at intensity levels great enough to produce shell vibration equivalent to that at vibration thresholds. He concluded that vibration sensitivity became increasingly important below 200 Hz and that low-frequency auditory sensitivity was replaced by vibration sensitivity below 64 Hz.

Other investigators have examined testudinate hearing using electrophysiological techniques, by implanting electrodes in the cochlea, VIIth nerve, or auditory brainstem. Wever and his coworkers measured the hearing of several species using cochlear potentials (*Pseudemys* [=*Chrysemys*] *scripta*, *Chrysemis picta*, *Clemmys insculpta*, *Testudo graeca*, and *Terrapine carolina*), showing that all species had good sensitivity in the range from 100-700 Hz. These studies supported Patterson's contention that the cochlea was the site of auditory perception. However, electro-

physiological measurements underestimated sensitivity in the best range and overestimated sensitivity at high frequencies relative to the behavioral measurements (Wever and Vernon 1956a, b, Patterson 1966, Wever 1978). The hearing of one species of sea turtle, the green sea turtle (*Chelonia mydas*), has been examined in air using cochlear potentials (Ridgway *et al.* 1969, Wever 1978). Their hearing resembled that of terrestrial turtles, despite the large difference in size and absence of a specialized tympanum. Best frequencies ranged from 150-700 Hz and were somewhat flattened at the high frequency end of the range (1000 to 2000 Hz) relative to Patterson's results. Ridgway *et al.* did not report estimates of the best sensitivities of the two turtles they tested; instead, they measured the sound pressure level required to produce a cochlear potential of 0.1  $\mu$ V. The greatest sensitivities they reported by this criterion were 39 and 53 dB re 20  $\mu$ Pa (65 and 79 dB re 1  $\mu$ Pa), respectively, at 300-400 Hz.

Patterson's turtles were tested at room temperature ( $\sim 22^{\circ}\text{C}$ ). The best sensitivity of the most sensitive animal in his study was 37 dB re 20  $\mu$ Pa (63 dB re 1  $\mu$ Pa; Fay 1988). However, this may not reflect the best performance of the species. The hearing of reptiles, including testudinates, is sensitive to changes in temperature. Wever (1978) measured cochlear potentials of a specimen of the wood turtle (*Clemmys insculpta*) over a  $20^{\circ}\text{C}$  range. The turtle's electrophysiological response increased 5-fold in this range, but the increase was not monotonic. Response increased slowly from  $8-15^{\circ}\text{C}$ , rapidly from  $15-20^{\circ}\text{C}$ , and reached an asymptote from  $20-30^{\circ}\text{C}$  (Wever 1978). These results suggest that air temperature must be considered when measuring hearing sensitivity of testudinates.

### Hearing Loss in Reptiles

There is no information on noise-induced hearing loss in tortoises. There is only one previous series of studies on noise-induced hearing loss in any reptile. Brattstrom and Bondello (1983) exposed Mohave fringe-toed lizards (*Uma scoparia*) to 500 s of taped dune buggy noise at 100 dB (flat-weighted SPL; integration method not specified), with most of the energy below 1000 Hz. The lizards were exposed 8 cm from the playback speaker. Auditory-evoked responses to 0.1 msec clicks were measured from implanted electrodes in the right telencephalon. Auditory-evoked responses showed a decrease in amplitude and an increase in latency relative to control conditions after exposure to clicks in the range from 75-85 dB. The click level at which responses recovered was not measured. These changes were detected shortly after exposure (*i.e.*, in the time it took to complete tests with the click stimulus). Brattstrom and Bondello did not use their data to estimate the magnitude of the hearing loss. Bondello exposed desert iguanas (*Dipsosaurus dorsalis*) to noise at levels of 115 dB (A-weighted SPL) for 1 and 10 hr, showing a decrease in cochlear potentials that recovered within 4 weeks of the test exposures. These lizards have been shown to

have relatively good sensitivity to sounds at typical desert temperatures (Werner 1972) in the range from 900-3500 Hz (Campbell 1969), with best sensitivities to test tones in the range from 15-25 dB SPL.

Brattstrom and Bondello (1983; Bondello and Brattstrom 1979) reported losses at exposure levels comparable to those that cause temporary loss in humans, laboratory animals, and birds. This result was unexpected because lizards have less auditory sensitivity than mammals and birds, and susceptibility to loss has been supposed to be a function of sensitivity. While this correlation may hold true within individuals of a species, there are no comparisons of susceptibility to hearing loss across widely-differing taxa, making it impossible to evaluate the results of Brattstrom and Bondello's preliminary study. In addition, Brattstrom and Bondello did not determine the extent of the shift in sensitivity in absolute terms nor were their characterizations of exposure adequate to permit comparison among studies.

### **Measurements of Hearing Threshold Using Auditory-Evoked Potentials**

For endangered and threatened animals, invasive measurements of electrophysiological potentials are undesirable. In unresponsive humans (babies, autistic children, *etc.*) and housepets, far-field auditory-evoked potentials (AEPs) are often used to measure hearing without significant risk to the subject. The most robust of these electrophysiological measures is the auditory brainstem response (ABR), which is generated by groups of neurons in the VIIth nerve, hindbrain, and midbrain when the ear is stimulated by sound. Far-field AEPs are measured by placing electrodes close to but not within these populations of neurons, usually with electrodes glued to the skin or placed subcutaneously. ABRs are used most often to evaluate hearing because they are relatively insensitive to subject state and small variations in electrode placement, are strongly correlated with changes in stimulus frequency and amplitude, can be used to determine threshold at many frequencies in a short time, occur in a wide variety of animals, and can be readily distinguished from electrical artifacts in the measuring system (Hall 1992). They appear at medium latencies after stimulus onset, typically 5-20 ms, following short-latency AEPs like the cochlear microphonic.

In order for an AEP to be detectable, there must be a high degree of synchrony in the firing of populations of neurons and the resulting signal must conduct well through surrounding tissues. These criteria may be met by activity of a number of structures within the ear and brain, ranging from the VIIth nerve to the midbrain. Precise maps of the structures generating such AEPs have not been made for testudinates and are unlikely to be developed for threatened and endangered species such as the desert tortoise because they cannot be developed without invasive electrophysi-

ological measurements. Far-field click-evoked ABRs and other AEPs have been elicited from all major groups of vertebrates, including one species of testudinate (Corwin *et al.* 1982, Bullock 1985), however, suggesting that the hearing of these animals could be measured using this technique as readily as that of humans and household animals.

Stimuli eliciting ABRs may be clicks, used to measure broadband hearing sensitivity; shaped tone-bursts, used to measure frequency-specific sensitivity; or more complex waveforms. The electrophysiological potentials elicited by these signals are measured as voltage differences between electrodes inserted on either side of the structure of interest. For the present study, activity in the cochlea, VIIth nerve and brainstem were of interest. Because these structures are close together in the tortoise, all were likely to contribute to any ABR measured from outside the skull.

Electrophysiological activity collected following a single stimulus presentation appears to be random noise. However, by collecting many samples in synchrony with stimulus presentations, then averaging, electrical activity unrelated to sound stimuli is eliminated, and a clear waveform emerges. Individual variability in the resulting ABR waveform is high, but a characteristic series of peaks can be identified after examining samples from a number of individuals of a given species.

ABR waveform peaks are generated as populations of neurons discharge simultaneously. As the intensity of a test stimulus decreases, amplitude of the ABR decreases as well. At low stimulus amplitudes, these peaks are no longer detectable. The least detectable waveform is used as an estimate of threshold, but tends to underestimate absolute sensitivity by a few dB because there are always other noise sources (electrophysiological and electrical) averaged into the sampled ABR. As test stimulus level decreases, latency from the stimulus onset to each ABR peak increases. Increasing latencies are therefore also an indication of decreasing sensitivity, although they cannot be used to infer thresholds at present.

While ABR waveforms have been characterized in great detail for humans and laboratory animals, relatively little is known about similar electrical activity in non-mammalian vertebrates. The only systematic measurements of far-field, whole brain potentials from testudinates were collected by Bullock and his coworkers (Corwin *et al.* 1982, Bullock 1985) from 3 red-eared turtles (*P. scripta elegans*; Figure 3). These investigators showed that both ABRs and frequency-following responses (AEPs that follow time-varying pressure precisely) could be elicited from the turtles, but they did not attempt to obtain measurements of threshold.

Generally speaking, if a detectable AEP is found, a subject animal can hear the test stimuli.

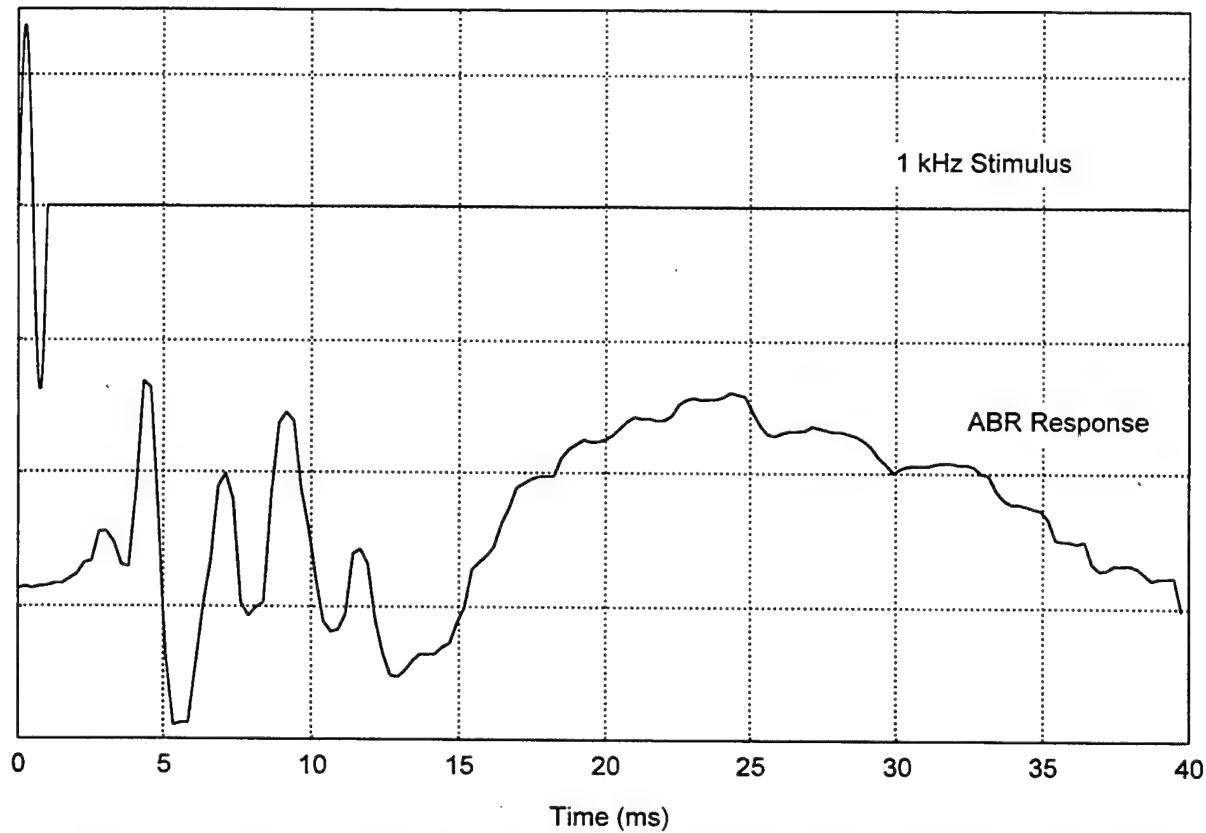


Figure 3. ABR waveform of a turtle (*Pseudemys scripta elegans*), bottom, elicited by one cycle of a 1 kHz tone, top (data from Corwin *et al.* 1982).

However, if no AEP is detected, the response may simply lack sufficient signal level to be detected above considerable electrophysiological and electrical ambient noise. Potentials from skeletal muscles and the heart are particularly likely to contaminate measurements.

In mammals, the least detectable ABR typically yields a somewhat higher, *i.e.*, less sensitive, estimate of threshold than conditioned responses (behavioral measures). Under optimal conditions, the difference may be as little as 5 dB. For animals that are difficult to condition, such as reptiles, it is unclear which method will yield the best estimate of threshold. As yet, no experiments comparing the two methods have been conducted in any reptile. The present experiments were designed to elicit ABR waveforms at specific frequencies in order to obtain an estimate of the tortoises auditory threshold function, but the main goal of the study is to look at relative changes in sensitivity after exposure to noise. ABR measurements are used routinely for this purpose in animal experiments.

The clicks and tone pips used to stimulate ABR waveforms are only a few milliseconds in duration.

They therefore differ substantially from the 200-500 ms tone bursts used to elicit responses in behavioral experiments. They are shaped by increasing level over 2-4 successive cycles at the beginning and end (onset and offset) of the tone pip to narrow the signal in the frequency domain while still stimulating a good ABR. Clicks consisting of a single cycle of a pure tone or a 1/2-cycle pulse (condensing or rarefacting) are used to elicit stronger responses over a broad band.

The tone pips and clicks used to elicit electrophysiological potentials are delivered in rapid succession (typically 10-50/s) over a period of minutes while averages are being collected. Therefore, although individual tone pips are very short, the sounds are roughly as perceptible as continuous tones.

At very low frequencies, tone pips must be of relatively long duration to contain even a few cycles. Unfortunately, long waveforms elicit poor ABRs and the resulting waveform is subject to distortion as other responses begin to overlap with the ABR. As a result, stimuli at frequencies below about 500 Hz are not normally collected for clinical applications. However, because turtle hearing is maximally sensitive at low frequencies (200-700 Hz), frequencies below 500 Hz were tested. Three measurements have been used with some success at low frequencies, (1) frequency-following auditory evoked responses (FFRs), (2) otoacoustic emissions (low-amplitude sounds returned by the outer hair cells of the ear upon stimulation with a sound), and (3) envelope following responses or modulation rate transfer functions, FFRs elicited by the amplitude or frequency modulation of a carrier wave at low frequency.

### **Behavioral Responses of Reptiles to Noise and Vibration**

Early observations of testudinate behavior in response to sounds had led naturalists to believe they were deaf (Patterson 1966, Carr 1952, Pope 1939). However, a series of experiments conducted early in this century using negative reinforcement techniques had shown that painted turtles (*Chrysemys*; probably *C. picta*) could hear whistles and bell tones (Andrews 1915). *Emys orbicularis* apparently could hear pipe organ and bell tones (Poliakov 1930), although some authors were unconvinced (Andrews 1915, Kuroda 1925). Chernomordikov (1958) and Karimova (1958) reported that turtles exhibited an 'electro-defensive reflex' (startle) to vibration, but not to purely acoustic stimuli (ringing bells, high frequency tones). These observations suggested that, while they can hear, testudinates do not have an acoustically-stimulated startle reflex.

Direct observations of testudinate response to impulsive noise are rare. Loggerhead sea turtles (*Caretta caretta*) were exposed to low-frequency impulses from a seismic survey airgun in a 300 x 45 m pen (O'Hara and Wilcox 1990). The absolute levels of the impulses from these stimuli were

not measured at the point of reception, but the level at one of the stimulus sources was 220 dB re 1  $\mu$ Pa (at 1 m) during the experiment. Loggerhead turtles were observed quiescent or resting in the net that held the sources regardless of the presence of impulses, but came within 30 m of the active sources significantly less often when they were swimming.

Although reptiles exhibit varied reactions to noxious stimuli, most of the behaviors are associated with direct attacks or other types of physical contact. Suboski (1992) reviewed the literature on responses to noxious stimuli that do not include contact, noting that freezing is a common and widespread response in amphibians and reptiles. Freezing is an abrupt cessation of motion that persists from a few seconds to hours; the more intense the stimulus, the more profound and protracted the inactivity. Frogs will allow themselves to be shocked to death as increasing stimulus levels trigger greater and greater inactivity (McGill 1960). Reptiles exhibit a wider range of responses to noxious stimuli, but freezing is still an important element in the repertoire. Lizards trained to avoid shock cease to respond with successive exposures, apparently lapsing into immobility as noxious stimuli are repeated. Only a few species escape reliably if provided an escape route after exposure to a species-appropriate noxious stimulus (Suboski 1992). Immobilization or suppression of activity can be induced artificially by physically restraining lizards, a phenomenon called tonic immobility (Gallup 1977). The duration of tonic immobility has been used as an estimate of the aversiveness of a stimulus in birds, lizards and amphibians. This response has not been studied in testudinates.

Testudinates are reported to be consistent in their responses to noxious (painful or aversive) stimuli—they drop to the ground, freeze, and withdraw head and legs into the shell (Ellis and Spigel 1966, Granada *et al.* 1965, Farris and Breuning 1965). Head withdrawal proved to be a reliable reflexive response to repeated electrical shock, allowing Patterson to conduct his behavioral study of hearing. However, negative reinforcement is too-little studied in testudinates to determine whether head withdrawal can be expected when the animal does not feel pain or contact.

### **Physiological Ecology of Desert Tortoises**

The energetic and water balance of the tortoise is very finely tuned (Nagy and Medica 1986). During the spring, when water is readily available, tortoises feed on succulent plants rich in water and salts but poor in protein and energy content. During this period they store water in the urinary bladder, but expend more energy than they take in. In late spring, they feed on matured and drying grasses, building up protein and fat reserves, storing energy reserves at the expense of some of their stored water. Their urine and blood become more concentrated. As the grasses dry and disappear, food becomes scarcer and summer heat stimulates them to estivate in burrows or

short hibernacula, conserving both energy and water as much as possible. The thunderstorms of the monsoon season bring them out to feed, drink and urinate, eliminating accumulated urates and salts and replenishing the energy reserves. By late fall, as temperatures begin to drop, they return to the ground to hibernate until the rains begin. Usually, this cycle of increases and expenditures leaves them with a positive energy balance at the end of each season, allowing them to grow and reproduce.

Desert tortoises survive by optimizing water balance, energy reserves (in the form of protein and fat), and activity. During the heat of the summer, urination or increased expenditure of energy could drain their reserves lethally, particularly during periods of drought, when they might go for periods of years without adequate food or water. Chronic increases in energy expenditure, while not always fatal, could impact growth, time to maturity, and reproductive output. Given this delicate balance, it is important to be able to accurately measure species-typical energy expenditure as a consequence of human-made perturbations. Metabolic rate is the standard measure of energy consumption by an animal, measured as the rate of oxygen consumed per unit of mass per unit of time. For poikilotherms like desert tortoises, metabolic rate is a function of (1) ambient temperature, (2) activity, (3) reproductive condition, and (4) food consumption.

Metabolic rate is typically measured by taking the difference between the amount of oxygen inhaled and that exhaled. Three variables influence the rate of oxygen transfer by the circulatory system. These are heart beat frequency (=heart rate), cardiac stroke volume (volume of blood pumped per beat) and the relative quantity of oxygen withdrawn from arterial blood by the tissues (usually expressed as the arterial-venous ratio [=a-v ratio]). Barthomelew and Tucker (1963) developed a formula to describe the relationship among these three variables and termed it the oxygen pulse or the amount of oxygen pumped by the heart in a single beat, a formulation that was later corrected by Gatten (1974). Usually, the oxygen pulse is quantified by measuring oxygen consumption and heart rate simultaneously, then correlating the average oxygen consumption and average heart rate during a measured interval, expressed as  $VO_2/\text{pulse-min}$ .

Most studies assume that the relationship among stroke volume, the a-v ratio, and heart rate remains constant across a variety of metabolic demands within a species (Barthomelew and Tucker 1963, Gleeson and Bennett 1985). If true, all of these measures should correlate directly (if not linearly) with oxygen consumption and with one another. The correlation can be used to estimate changes in metabolic rate by measuring changes in heart rate. However, to use heart rate to estimate metabolic rate one must calibrate the relationship, as the relationship between heart rate, cardiac stroke volume and a-v ratio can vary greatly among species. Gatten (1974)

found that for the pond slider (*Pseudemys scripta*) increase in heart rate accounted for 6% of the increased oxygen transport, while for the western box turtle (*Terrapene ornata*) it accounted for 27% of the observed change.

Since heart rate is an indication of increased oxygen consumption, it can also be used to measure the time needed to recover from exercise or a startling event, and the energy expended during the reaction. Gatten (1988) measured heart rate responses of the common snapping turtle (*Chelydra serpentina*) to handling and to exercise. The time required for heart rate to decline to the standard rate (=average daily heart rate) after handling was significant, on the order of 4-6 hours.

Desert tortoises are active in the temperature range from 11-38°C (Vaughn 1984, Wirt 1988), although measurements of their internal body temperature show a narrower range in active animals, from 26-37.6°C (McGinnis and Voigt 1971). In general, they regulate their temperature by the timing of emergences from the burrow (crepuscular during the summer; at midday during the fall and early spring), and by virtue of their heavy protective carapace. As a general rule, tortoises begin to enter hibernation when ambient temperatures drop below 20°C (Holm 1989).

## METHODS

### Animal Handling and Husbandry

#### Permits

The fourteen desert tortoises used in these experiments were collected from a known population in Barstow, California, at the site of a proposed development. The collection and experimentation was allowed under U.S. Fish and Wildlife Service Regional Blanket Permit PRT 702631, Subpermit ED-AFB, recovery subpermit to take the desert tortoise (*Gopherus agassizii*). This permit allowed a maximum of three mortalities due to captive maintenance or experimental conditions. The tortoises were collected 18-19 and 25-26 February 1995 by personnel from Edwards Air Force Base Environmental Management Office, which was also responsible for finding adoptive homes for the tortoises at the end of the study. Tortoise husbandry and experimental handling were monitored by the HSWRI Institutional Animal Care and Use Committee (IACUC) according to the Guidelines for Use of Live Amphibians and Reptiles in Field Research, American Society of Ichthyologists and Herpetologists (1987) and Schaeffer *et al.* (1991).

#### Animal care

The tortoises were transported to HSWRI within 24 hr of capture. They were kept in separate 1.22 x 2.44 m (4x8') enclosures, allowing each animal a total of 2.97 m<sup>2</sup> (32 ft<sup>2</sup>) of surface area. Each enclosure was filled with 2.54-5.08 cm (1-2") of commercial compressed vegetable matter bedding (Mountain Meadows Pet Products) and was lined with a plastic tarpaulin for ease of cleaning. Each tortoise was given a 46 cm (18") black plastic trash pail with one side cut off to use as cover (hereafter, burrow).

The tortoises were kept under commercial reptile lights on an artificial summer light cycle, 16 hours on and 8 hours off. The on and off times of the lights in each pen were staggered slightly, so that light levels in the room increased and decreased over a period of an hour. The room was kept between 28-34°C and 18-30% humidity using commercial airconditioners and de-humidifiers. These were noisy enough that they had to be mounted outside the building, connected to the room by several ducts. After this modification, sound levels in the room averaged 45-48 dB (L<sub>eq24</sub>). Ceramic, infrared heat lamps were provided at one end of each enclosure, but were not used often.

When first introduced, some tortoises ate well immediately. Those that didn't were coaxed to eat by hand feeding. A few were lethargic and unwilling to eat. These were given lactated Ringer's solution subcutaneously to hydrate them. All of the tortoises had internal parasites on arrival. Nematodes and cestodes were treated with Panicure; strongyloids and other enteric prokaryotes were treated with Flagyl. One individual had mild symptoms of upper respiratory tract disease (URTD), and it was given a course of Baytril. The symptoms did not recur. Tortoises were x-rayed on entry to look for lung congestion, stones, and eggs. Eggs were difficult to detect in a dorsal x-ray or by palpation, however - although no eggs were detected when the tortoises arrived, one individual later proved to be gravid (LL52).

The tortoises were fed once daily with a mixture of greens (kale, Romaine lettuce, grass, spinach, dandelion greens), cactus leaves, fruit (apples), and vegetables (broccoli, squash, cauliflower) covered with the recommended ration of commercial iguana mash (Smithsonian Institution formulation; Table I). Periodically, they were fed cut grasses to increase dietary fiber. Enclosures were cleaned daily to remove urine-soaked patches, dried out food, and feces. Location and amount of urine and feces were noted. Once per week, tortoises were given water *ad libitum* and once per month they were bathed to clean off accumulated fecal material.

Each tortoise had its own food dish and other paraphernalia. Caretakers changed latex gloves or disinfected their hands before handling each successive individual to prevent the spread of disease or parasites.

#### Noise levels at the site of capture

During the first two days of the capture effort a Larson-Davis 820 community noise monitor (LD820) was deployed to record ambient noise levels and any transient events exceeding 80 dB (A-weighted maximum sound pressure level,  $L_{max}$ ). The microphone was fitted with a 15 cm open-pore foam windscreen that eliminated wind noise at speeds below 15 kt, and was deployed on a pole 1.2 m from the ground in an area removed from the capture efforts. Daily averages were also measured for the two days of the capture effort.

Sound transmitted into the burrow from aircraft overflights and sonic booms could not be measured. However, a brief effort was made to determine the relative transmission loss across frequency inside a desert tortoise burrow. A single desert tortoise burrow was exposed to white-noise bursts transmitted from a loudspeaker mounted at an oblique angle over the mouth of the burrow. Burst levels above ground were measured with the LD820. Levels underground were measured with an ACO Model 7013 1/2" Type 1 microphone powered by an ACO PS9200 power supply was fed into Casio model DA-7 digital audio tape recorder (DAT). The ACO microphone

Table I: Dietary supplement given per day per tortoise

Tortoise Length (cm)	Amount (level teaspoons)
< 5	0.25
5-8.9	0.38
9.0-10.8	0.5
10.9-12.7	1.0
12.8-15.2	1.5
15.3-17.8	2.0
17.9-20.3	3.0
20.4-22.9	4.0
23.0-24.1	4.5
24.2-25.4	5.0
25.5-27.9	5.75
> 27.9	6.0

was covered with a 5.5 cm foam windscreens and attached to the end of a 100 cm long, 2 cm diameter aluminum pole. The microphone was mounted on the pole with the gridcap facing back, that is, towards the opening of the burrow, and was fed 80 cm into the tortoise burrow. Both the ACO microphone and the microphone for the CEL system were calibrated with a Gen Rad Omnical 1986 calibrator.

### Sound Simulations

#### Simulation of subsonic aircraft noise

Simulated jet overflights were generated in a sound isolation chamber at HSWRI, an Industrial Acoustics Company Model 1202A chamber (IAC chamber). The sound isolation room reduced ambient noise and vibration in the building significantly except at very low frequencies (< 30 Hz). Chamber features included double-wall (room-within-a-room) construction with 10.2

centimeters (cm; 4 in) thick panels separated by 10.2 cm of air space (Figure 4). The inner room was mounted on vibration isolators. Inside dimensions were 1.93 meters (m) wide by 1.83 m long by 1.98 m high. Signals were conducted in and out of the room via a connector panel fitted with isolated BNC connectors on the outside and inside of the room. A 4-cm diameter hole through both walls served as a feedthrough for cables.

A TEAC RD-101T digital audio tape (DAT) deck was used to deliver low-altitude jet overflight recordings in the sound isolation chamber. The TEAC DAT is a quantitative recorder with adjustable calibrated input levels and a fixed output level. It also has a flat frequency response down to DC (0 Hz). Output of the DAT deck was sent to the U.S. Air Force Aircraft Noise Simulation System (ANSS), consisting of a Bose 802-C speaker system controller, and a Crown Macro-Tech 1200 power amplifier driving two Bose 802 Series loudspeakers connected in parallel. Figure 5 shows a block diagram of the sound generation system. Equalization and noise reduction was accomplished by driving the system with the HSWRI TEAC DAT deck, which has a dynamic range  $> 80$  dB and flat response down to DC. Both speakers were suspended face downward in the sound isolation chamber by elastic cords attached to a framework near the ceiling. Elastic cords were used to decouple speaker vibration from the chamber as much as possible. The speaker cables (low-resistance Monster-type cables) were routed through a feedthrough hole to the power amplifier.

Table II summarizes the contents of the tape used to deliver subsonic noise exposures. The tape was designed to provide a worst-case scenario for low-altitude overflights in the absence of specific information about planned operations. The rate and intensity of overflights was determined from worst-case measurements collected in desert training areas on the Barry M. Goldwater Air Force Range in Arizona (Bowles *et al.* 1995). The aircraft types were chosen to maximize variability, as playbacks that always sound the same cause unnaturally rapid habituation.

Overflight digitization was performed on a Pentium PC equipped, appropriately enough, with a Turtle Beach Systems Tahiti sound card and a Systems Wave SE sound recording and editing package. Each overflight was recorded to a separate file. Maximum dynamic range for the simulated overflights was achieved by setting the loudest overflight so that the peak sound pressure level (SPL) was just below the level that caused an overflow in the 16-bit analog-to-digital (A/D) converter. The Wave SE software was used to fade the overflight sound from zero amplitude (onset) and to zero amplitude (offset). Care was taken to ensure that fade-in of the overflight did not reduce the peak overpressure. Overflights were faded-out over about two seconds to eliminate dropout transients. The 20 overflights were scripted in random order using

Signalogics Hypersignal-Acoustic macro language, with one overflight for every two minutes of the 40 min tape. The overflights were generated by the Tahiti board and recorded onto the TEAC RD-101T DAT. The resulting tape was played for each experiment.

Sound metrics of the overflights generated in the sound isolation room were collected using the LD820 (Table II). A  $1.9 \times 0.81 \times 0.38$  m pen was constructed in the chamber and filled with pressed-vegetable bedding. The LD820 microphone was placed at the center of the holding pen at a point equidistant from both speakers and 0.095 m above the bedding, roughly the height of a tortoise's head. Playback levels were adjusted until the most intense overflights reached 110 - 120 dB peak fast sound pressure level ( $L_{max}$ ).

Simulated overflight levels were measured with the LD820 during actual experiments. Sound metrics collected for the overflight events were C-weighted level equivalent ( $L_{eq}$ ),  $L_{max}$ , sound exposure level (SEL), and unweighted peak level. An event was recorded each time the pre-set threshold on the device was exceeded. Event durations and the intervals between were also measured. The thresholds set were as follows: fast threshold 1 of 70 dB, threshold 2 of 80 dB, peak threshold 100 dB C-weighted (dBC), peak unweighted threshold of 120 dB, and a hysteresis of 6 dB.

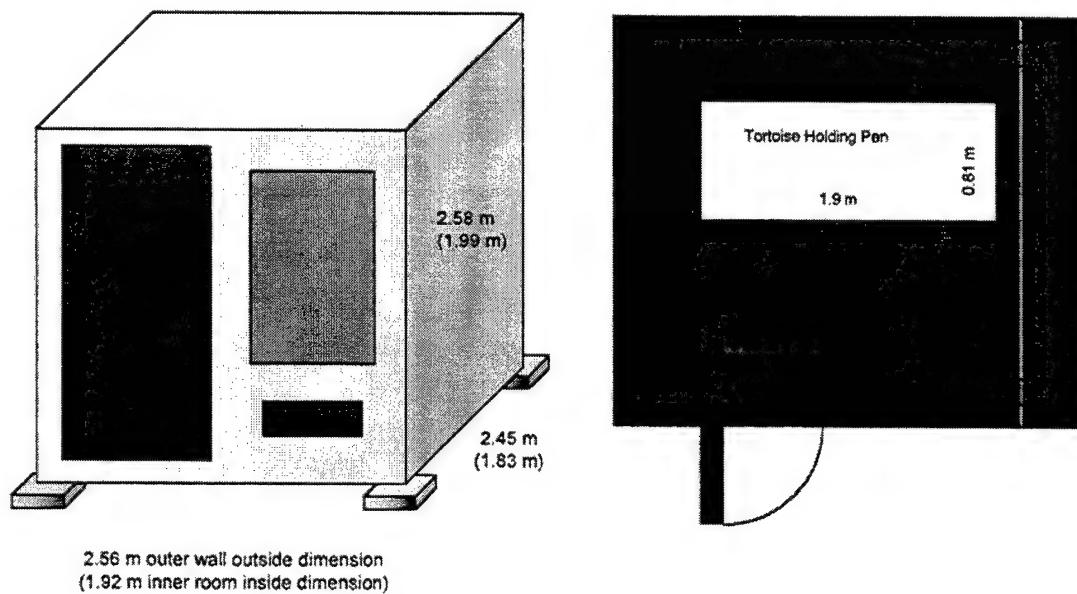


Figure 4. Diagram of IAC sound isolation chamber used for playback experiments with sub-sonic aircraft noise. The location of the tortoise pen is shown.

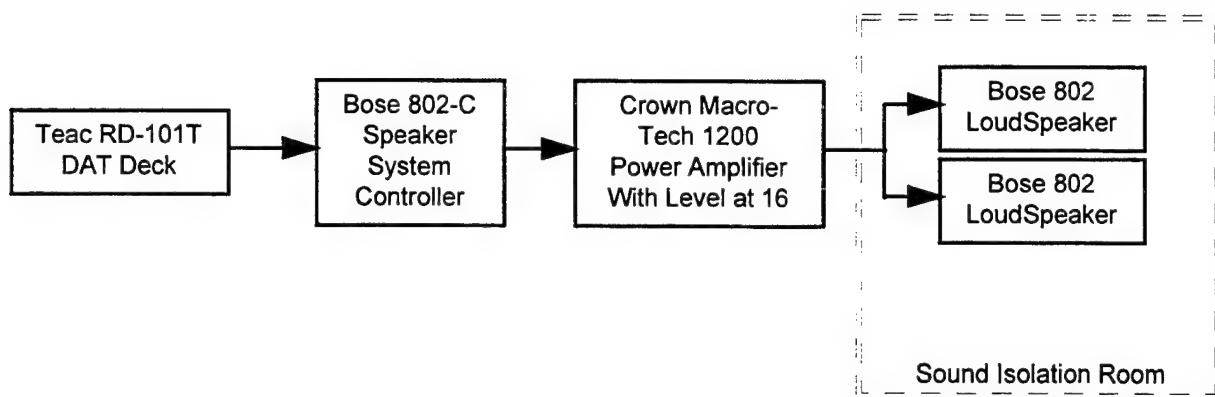


Figure 5. Sound generation system block diagram.

Table II. Simulated jet overflight playback parameters measured in the HSWRI sound isolation room.

Overflight Number	Jet	Time (mm:ss)	Duration (seconds)	C- Leq (dB)	C- Lmax (dB)	C- Peak (dB)	Uwpk dB	CSEL (dB)
1	F-16	00:00	09.4	90.8	100.8	111.3	112.0	100.5
2	F-16	02:13	11.1	98.4	113.3	122.6	124.8	108.8
3	F-16	04:28	20.1	87.2	97.5	106.6	107.9	100.2
4	F-16	06:53	18.8	81.9	89.6	98.5	101.9	94.6
5	B-1B	09:14	24.1	100.4	109.4	120.0	119.9	114.2
6	B-1B	11:42	21.8	100.9	109.9	119.8	120.6	114.2
7	F-4	14:07	28.3	97.2	105.3	115.5	116.9	111.7
8	F-4	16:38	17.9	89.3	97.6	109.3	111.5	101.8
9	F-4	19:00	13.8	100.3	109.1	119.1	121.3	111.6
10	F-16	21:17	09.0	89.6	96.8	106.3	109.2	99.2
11	F-16	23:30	22.3	89.2	100.1	109.1	110.1	102.7
12	F-16	25:55	13.8	85.2	92.1	101.3	103.7	96.6
13	A-10	28:13	17.2	93.4	105.8	115.8	117.7	105.7
14	A-10	30:32	15.8	87.3	92.5	101.2	104.3	99.3
15	A-10	32:52	18.9	87.3	95.1	105.1	106.2	100.1
16	A-10	35:13	23.9	87.8	97.1	106.6	108.1	101.6
17	A-10	37:40	18.1	98.6	111.3	121.1	123.2	111.2
18	A-10	40:00	22.4	87.0	92.9	101.8	104.8	100.6
19	F-16	42:27	09.9	98.4	109.1	120.4	120.5	108.4
20	F-16	44:39	18.3	98.8	115.6	124.3	126.1	111.4

### The HSWRI Impulse Noise Test Facility

An Impulse Noise Test Facility (INTF) was constructed at Hubbs-Sea World Research Institute (HSWRI) to study animal auditory and non-auditory physiological responses to impulse noise. It consisted of a test chamber to deliver stimuli and a computer to condition the signal and control the tests.

System description. The Impulse Noise Simulator (INS) chamber was made of 2.54 cm (1 in) thick marine-grade aluminum. Internal chamber dimensions were 0.46 m (1.5 ft) high, 1.13 m (3.67 ft) wide and 2.03 m (6.67 ft) long. Six large speakers were installed in the ceiling of the chamber and four small speakers in the front door (Figure 6). A connector panel in the front door allowed instrumentation to be connected to external devices. During the tortoise experiments, the chamber was equipped with a flat-panel infrared heating element and a fluorescent broad spectrum light in the ceiling between the large speakers. A video camera bracket was mounted to the front door of the chamber. Neoprene gaskets sealed the connectors, speakers, and doors to provide an airtight seal when the doors were closed, preventing air from entering and leaving the chamber, allowing adequate pressure to develop when the speaker diaphragms moved.

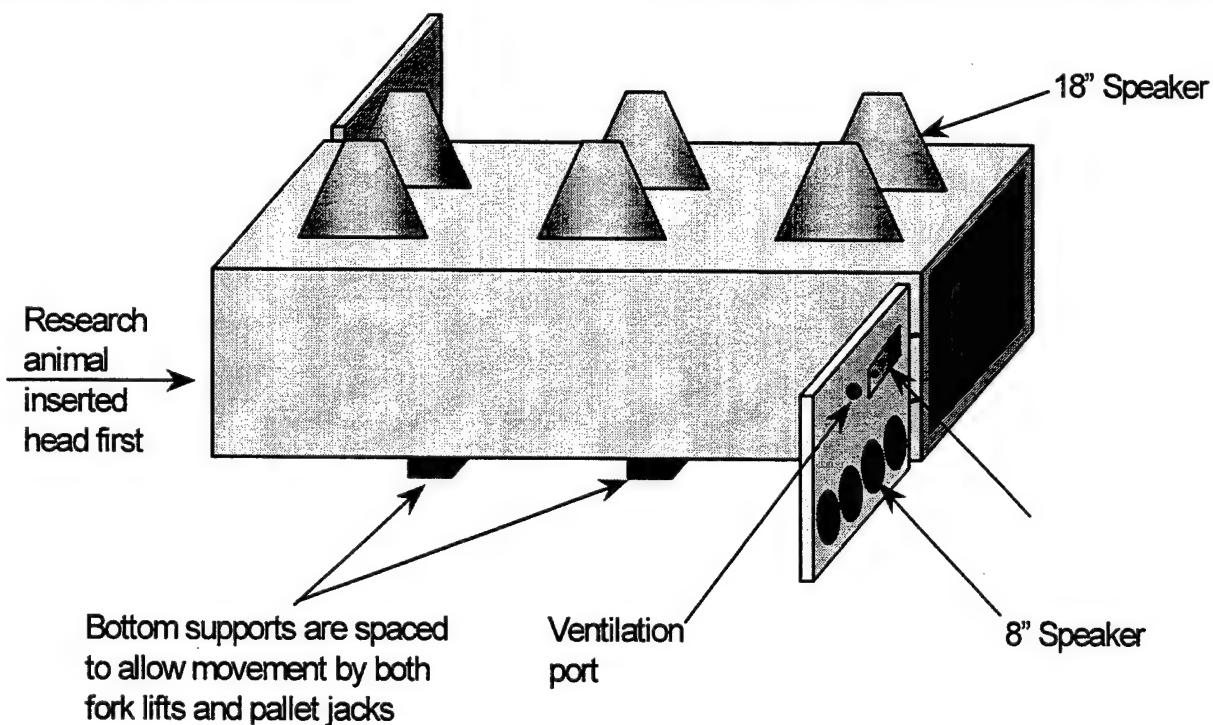


Figure 6. Diagrammatic view of Impulse Noise Simulator.

A computer equipped with a sound card controlled impulse generation. Analog-to-digital (A/D) and digital-to-analog (D/A) signal conversion was performed by the sound card. Stored digital samples of the sonic boom waveform were sent to the D/A converter for signal generation; the boom pressure wave was received by a carrier microphone inside the test chamber for digitizing and storage. The microphone output was low pass filtered with a cutoff frequency of less than half of the sampling rate of the A/D converter to prevent aliasing.

D/A output was sent to a smoothing filter with a low-pass cutoff frequency of less than half the sampling rate, which was used to reduce step-like quantization noise. The smoothing filter output was sent to an electronic crossover network, which took the input signal, high-pass and low-pass filtered it in parallel, and sent the resulting low-frequency signal to the power amplifiers.

Signal processing and waveform fidelity. Each of the components of INS distorted the input waveform to some degree. There was also spatial variation in the sound field resulting from the physical characteristics of the test chamber, a small closed volume with distributed sound sources (the speakers). The waveform distortion had to be corrected to produce the distinctive N-wave of a sonic boom. This was accomplished by pre-distorting the signal to account for the system transfer function, the ratio of the cross-spectrum of the output and input signals to the auto-spectrum of the input signal.

Ideally, a unit impulse function is used as the input signal to generate the system transfer function. However, in practice, white noise is used as the input signal in place of an impulse because the power spectra of the two are alike - constant across all frequencies - and white noise presents fewer signal processing challenges. The cross-spectrum of output and input signals is used in the transfer function estimate since it preserves the phase response of the system.

An ideal system transfer function is constant across all frequencies with linear phase. It is a property of such a transfer function that there is no relative amplitude difference in the frequency content of the signal and a constant group delay, that is, no waveform distortion. Therefore the goal of correcting waveform distortions was to equalize, or make equal at all frequencies, the system transfer function.

Perfect equalization could only be accomplished in one small area of the chamber due to spatial variation in the sound field in the test chamber. This was called the reference location (a sphere with a diameter of 24 cm). Waveform distortions were corrected by collecting the system transfer function and then creating a filter that was essentially the inverse of the system transfer function. The transfer function was measured by placing a microphone at the reference location

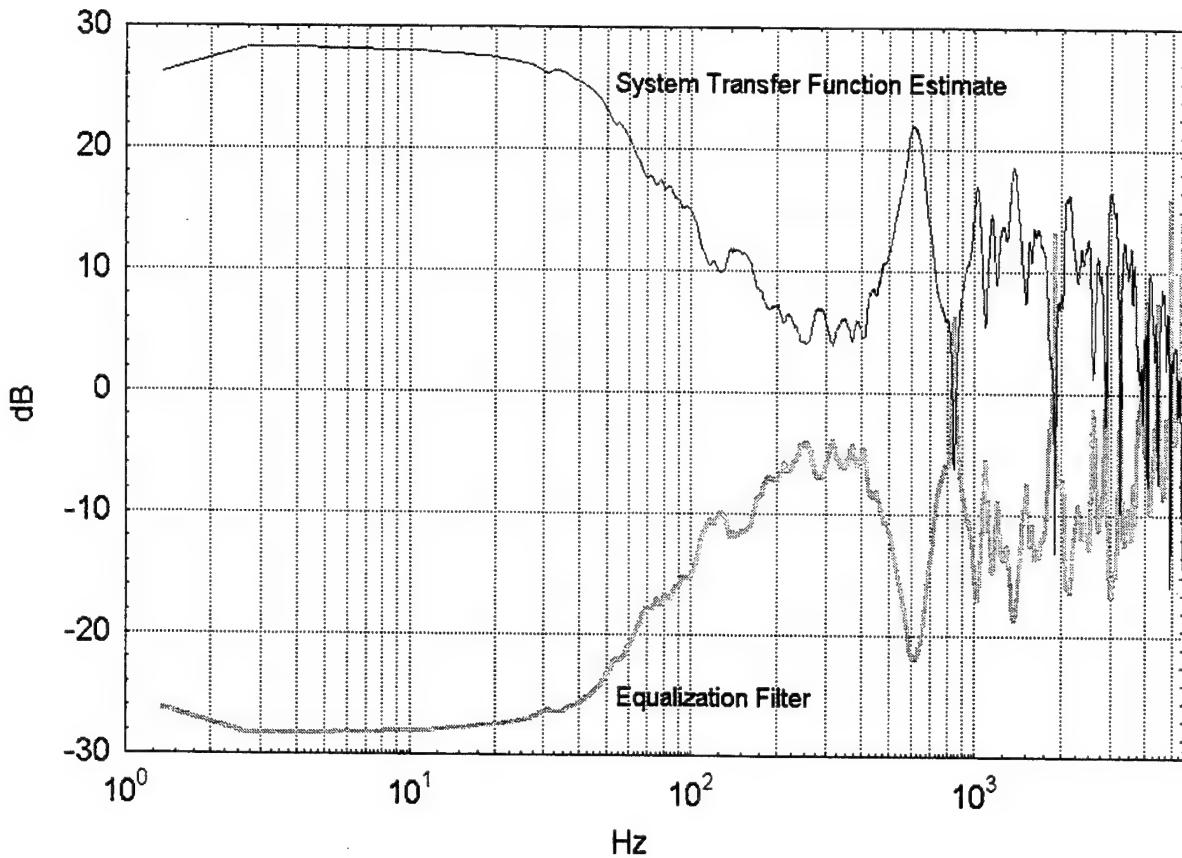


Figure 7. Impulse Noise Simulator transfer function and equalization filter.

to record the output signal while white noise was played through the system. The transfer function was then used to calculate the equalization filter (Figure 7). During experiments, a sonic boom waveform was pre-distorted by passing it through this filter prior to sending it through the system. This pre-distorted waveform passed through all the elements in the signal path, producing the desired waveform at the reference location.

*System components.* Figure 8 shows a block diagram of the system signal chain. Samples of the signal waveforms were stored in files on the computer hard drive. Signalogic's Hypersignal-Acoustic (HSA) software package was used to control the 16-bit Tahiti board D/A and A/D converter operations. The input signal was sampled at 11,025 Hz. One channel of a Stanford Research Systems SR640 Dual Channel Filter was used as an input filter. It was an 8-pole, 6-zero elliptical design with 0.1 dB maximum passband ripple and amplification available in 10 dB steps. The other filter channel was used as the anti-aliasing filter for the microphone signal. A cutoff frequency of 5 kHz was used on both channels.

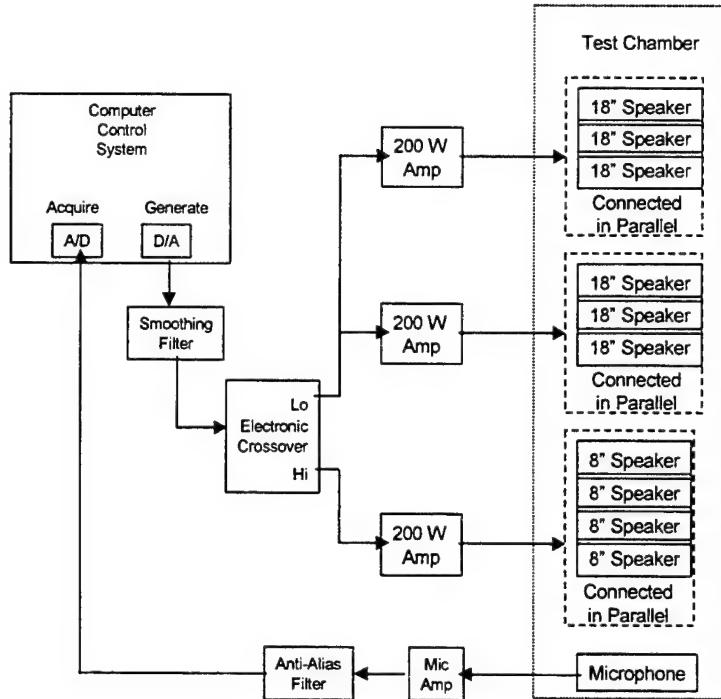


Figure 8. Impulse Noise Simulator signal chain block diagram.

The electronic crossover was a TDM Design model 24CX-2. It provided 24 dB per octave attenuation and had an adjustable crossover frequency. The crossover frequency was set to around 410 Hz.

B&K Components Ltd. EX4420M 200-watt mono power amplifiers drove the low impedance parallel speaker combination. Two of the amplifiers drove a parallel combination of three large speakers and the other drove the parallel combination of four small speakers. The 18 inch diameter speakers were JBL model 2245H and the 8 inch speakers were JBL model 2118H. All of the speakers had an impedance of 8 ohms.

The microphone was a Brüel and Kjær Microphone Carrier System Type 2631 which had a flat frequency response down to DC (0 Hz). This DC capability eliminated the problem of trying to separate the microphone frequency response from that of the rest of the system during equalization.

#### Sonic boom parameters

The desired simulated sonic booms were idealized N-waves 120 ms (milliseconds) in duration, with the worst-case parameters expected for the F-22 (parameters were selected in consultation

with Parsons Engineering Science [memorandum of June 8, 1995 from Areg Gharabegian] and USAF representatives).

Carpet booms were calculated to have a worst-case peak overpressure of 7.52 pounds per square foot (psf). Other parameters were selected to insure that the simulated sonic booms had maximal spectral energy in the range heard best by tortoises. One of the biggest challenges in generating the simulated sonic booms was the production of sufficiently rapid rise times while still achieving high signal levels. Rise time was the interval between N-wave onset and the first peak (Figure 9). Simulated booms with fast rise times produced resonances within the aluminum INTF chamber, producing an odd 'ring' in the simulated waveform. Reducing this ring while still maintaining short rise time proved to be a constant challenge, particularly once the animal had been inserted into the chamber. Sonic booms from fighter aircraft usually last 100 ms; in the INTF, booms of 120 ms duration proved easiest to produce. This was therefore chosen as the duration of simulated sonic booms.

The maximum peak overpressure that could be achieved by the INTF for fast onset times (< 1 ms) without significant distortion was 6 psf (Table III; 6 psf = 287.3 Pa = 143 dB peak flat-weighted SPL). This was within 2 dB of the target 7.52 psf level (= 145 peak flat-weighted SPL). In order to produce a cumulative exposure somewhat in excess of the anticipated level, tortoises were exposed to two 6-psf booms in quick succession (separation of 3.1 s; cumulative flat weighted sound exposure level of 119 dB flat-weighted SEL vs. 115 dB for the anticipated F-22 sonic boom).

The F-22 was also expected to produce focused sonic booms at levels that could reach 25 psf (= 155 dB peak flat-weighted SPL; 126 dB flat-weighted SEL). The rise-time and waveform of such a focused sonic boom could not be predicted. Under ideal conditions, focused booms have a U-shaped waveform. However, the focal region is very small and considerable distortions occur if (1) a receiver is just outside the focal region or (2) if atmospheric turbulence introduces distortions. It was not within the scope of this study to examine a wide range of possible boom waveforms. Therefore, it was felt that test stimuli should have a consistent waveform. In order to present a cumulative level in excess of 25 psf, tortoises were exposed to 10 6-psf booms in rapid succession (3.1 s interval), for a cumulative flat-weighted SEL of 133 dB, slightly in excess of the expected worst-case SEL of the focused boom.

It was recognized that this procedure did not simulate real focused booms perfectly. However, rise time was as important as absolute level in producing hearing damage because fast rise times produce greater high-frequency energy in a sonic boom. Spectral content in the center of an

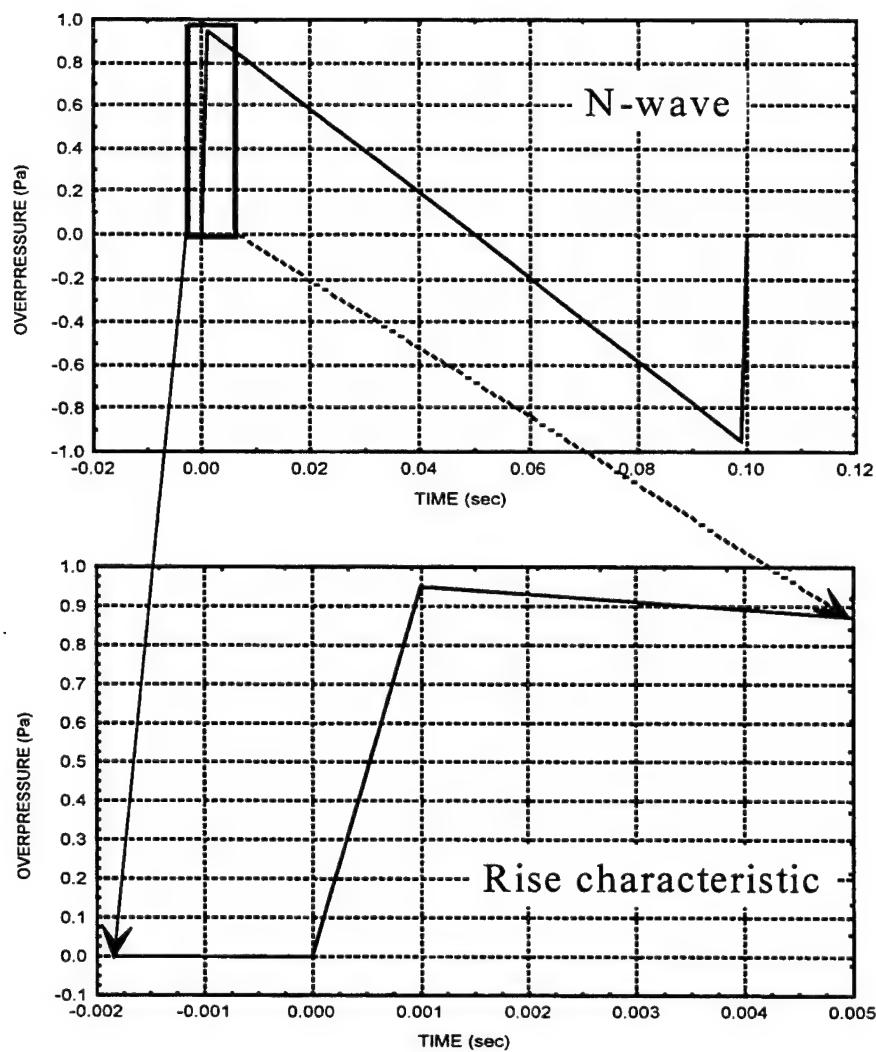


Figure 9. N-wave characteristics. In this example, the N-wave has a duration of 100 ms, a rise time of 1 ms, and a peak overpressure of 0.95 Pa (93.5 dB).

Table III. Estimated exposure levels of sonic booms and cumulative sound exposure level of repeated exposures. Sound pressure levels given in flat-weighted dB re 20  $\mu$ Pa. Sound exposure levels given in flat-weighted dB re 20  $\mu$ Pa $^2$  · s. Estimates of sound exposure level presume a sonic boom duration of 120 ms, except for the 10.5 psf boom, which had a duration of 130 ms.

# Booms/ Peak Level (psf)	Peak Pressure (Pa)	Peak SPL (flat-weighted dB)	Sound exposure level (flat-weighted dB)
1@ 0.25 psf	12.0	116	86
1@ 0.50 psf	23.9	122	92
1@ 1.00 psf	47.9	128	98
1@ 2.00 psf	95.8	134	104
1@ 3.00 psf	143.6	137	107
1@ 4.00 psf	191.5	140	110
1 @ 6.00 psf	287.3	143	113
1 @ 7.52 psf	360.1	145	115
1@ 10.50 psf	502.7	148	118
2 @ 6.00 psf	287.3	143	119 (cumulative)
1 @ 25.00 psf	1197.0	156	126
10 @ 6.00 psf	287.3	143	133 (cumulative)

animal's hearing range is recognized as an important predictor of hearing loss due to impulse noise (CHABA 1992). Therefore, presenting a series of exposures was preferable to relaxing the rise-time requirement. It was recognized that this exposure would produce a conservative estimate of the potential for loss because repeated exposures are more likely to result in TTS than single exposures. If any TTS were detected as a result of the exposures, the cause would have to be ascertained by further experimentation. However, both the anticipated peak and cumulative exposure of the focused sonic boom were below sonic boom levels used in field experiments conducted by Nixon *et al.* (1968), which resulted in no detectable TTS. The simulations were therefore a reasonable starting point for experimentation.

Parameters of the simulated sonic booms used in these experiments are given in Table III. The waveforms of an ideal and simulated N-wave with 120 ms duration, 0.4 ms rise time and 3 psf peak overpressure are shown in Figure 10. The figure shows the simulated boom with no animal

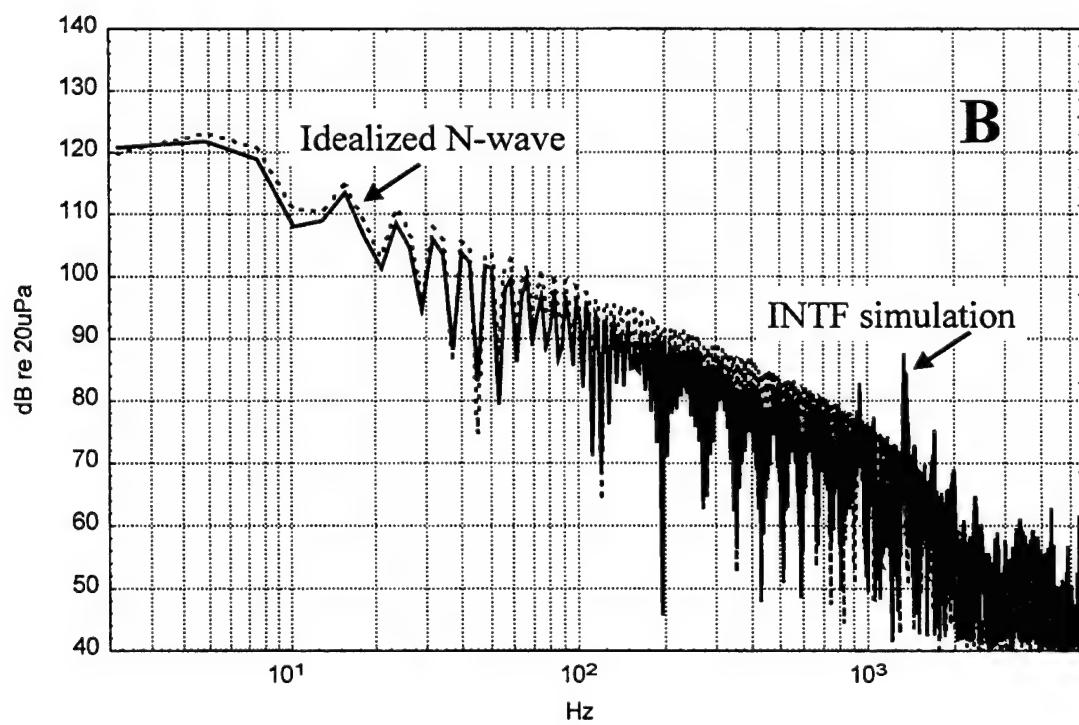
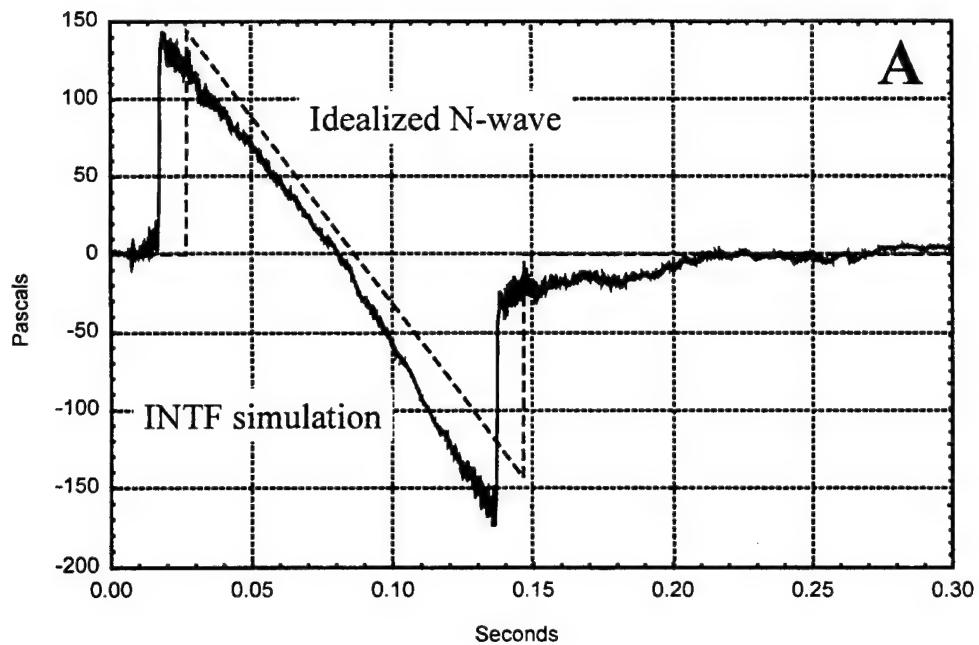


Figure 10. Ideal and simulated waveforms (A) and spectra (B) for an 120 ms duration, 0.4 ms rise time, 3 psf peak overpressure N-wave.

in place. In practice, placement of a tortoise on a restraint board in the chamber resulted in some distortion. Variation was also introduced by placing the receiving microphone beside the tortoise instead of at the point of calibration (where the tortoise's head was placed). For this example N-wave, the mean peak overpressure (the average of the maximum positive value of the N-wave) was 3.16 psf (151.49 Pa), corresponding to sound exposure levels (SEL) of 123.2 dB re 20 uPa<sup>2</sup>s for the ideal N-wave and 123.5 dB re 20 uPa<sup>2</sup>s for the INTF wave. The corresponding spectra are shown in Figure 10b. Spectra were calculated using 4096 point fast Fourier transforms (FFT; frequency bin width = 2.692 Hz). The peak level for the ideal and simulated spectra were 123.1 dB and 123.3 dB, both in the spectral band centered on 5.383 Hz (5.383 Hz bin).

A script was created for the simulated sonic boom playback experiments using the Signalogics Hypersignal-Acoustic macro language. Scripts used during hearing experiments allowed a specifiable number of 6 psf, 0.4 ms rise time, 120 ms duration booms to be generated in succession at 3.1 s intervals. The scripts used during the behavioral experiments were more complex. Tortoises were exposed to single booms at 0.25, 1, and 6 psf at varying intervals in an effort to measure level-specific responses. The first script was used for experiments in which the tortoise was equipped with an activity monitor only (no heart rate monitoring via a tether; see below). Script 1 (Table IV) delivered two sets of 10 booms with exposures beginning at 10:30 and at 19:30. Each set consisted of four 3-psf and five 1-psf simulated booms with 0.4 ms rise times. The last boom in each set had a 10 psf peak overpressure, 5 ms rise time, and 130 ms total duration. The booms were separated by intervals of 10 min. An additional series of exposures was conducted using the tethered heart-rate recording system. Script 2 delivered two sets of 10 booms, one beginning at 10:00 and the second at 12:30 (Table V). Each set consisted of 4, 2, 1, 0.5, and 0.25 psf, 0.4 ms rise time booms and delivered in quick succession (3.1 s between).

### **Measurement of Hearing and Vibration Sensitivity**

#### **Hearing 200 Hz and above**

Two types of stimuli were delivered during the ABR measurements, tone pips and clicks. As described in the introduction, the tone pips were only a few milliseconds in duration and were shaped to smooth the onset and offset. As frequency decreased, the number of cycles in the tone pip decreased, but had to consist, at minimum, of 3 cycles (1-2 onset cycles, 1-2 cycles at the desired level, 1-2 offset cycles). Table VI summarizes the structure of the tone pips used in the experiments; note that at low frequencies, the tone pip became longer and longer.

Table IV. First sonic boom playback protocol (script 1).

Time	Interval (min)	Peak Overpressure (psf)	CSEL
1030	-	4	109
1040	10	1	99
1050	10	4	109
1100	10	1	99
1110	10	4	109
1120	10	1	99
1130	10	1	99
1140	10	4	109
1150	10	1	99

Table V. Second sonic boom playback protocol (script 2).

Time	Interval (min)	Peak Overpressure (psf)	CSEL
1030	-	1	95
1040	1	2	102
1050	1	4	109
1100	1	6	113
1110	1	0.5	88
1120	1	1	95
1130	1	2	102
1140	1	4	109
1150	1	6	113
1200	1	0.5	88
1930	-	6	113
1940	10	4	109
1950	10	2	102
2000	10	1	95
2010	10	0.5	88
2020	10	6	113
2030	10	4	109
2040	10	2	102
2050	10	1	95
2100	10	0.5	88

Narrowband tone pips were delivered to determine the frequency-specific responses to noise. An example tone pip is shown in Figure 11. The only frequency component of the tone pips that had a good signal to noise ratio was the narrow peak at the desired stimulus frequency.

Tortoises were also tested with clicks (Figure 12), consisting of a single positive half-cycle (compressive click). These clicks had relatively constant energy throughout the tortoises' hearing range (Figure 12, bottom). Clicks were delivered to measure (1) the form of the species-typical ABR, (2) changes in ABR amplitude with varying presentation rate, and (3) the variations in responsiveness with temperature and noise exposure.

Stimulus levels were measured using the method accepted for psychophysical experiments (in terms of RMS SPL of individual tone pips or clicks). The metric requires some justification in this case because both the clicks and tone pips were of such short duration. In humans and laboratory animals, brief signals become louder and louder, and therefore more and more audible, as duration increases up to 300-1000 ms. Longer signals have the same loudness, regardless of duration. Below around 300 ms, perceptual level decreases at a substantial rate; estimated at 5 dB per halving of stimulus duration. Therefore, the longer, low frequency tone

Table VI. Characteristics of tone pip stimuli used to elicit ABR measurements.

Stimulus Frequency	Duration (ms)	# Cycles	Onset/ Steady State/ Time (ms)	Window Length (ms)	Repetition Rate (/s)
Click	5	0.5	-	20	11.3-29.3
250 Hz	20	5	8/4	30	29.3
500 Hz	15	7.5	6/3	30	29.3
1000 Hz	6	6	2/2	20	29.3
2000 Hz	5	10	2/1	20	29.3
3000 Hz	5	15	2/1	20	29.3
4000 Hz	5	20	2/1	20	29.3

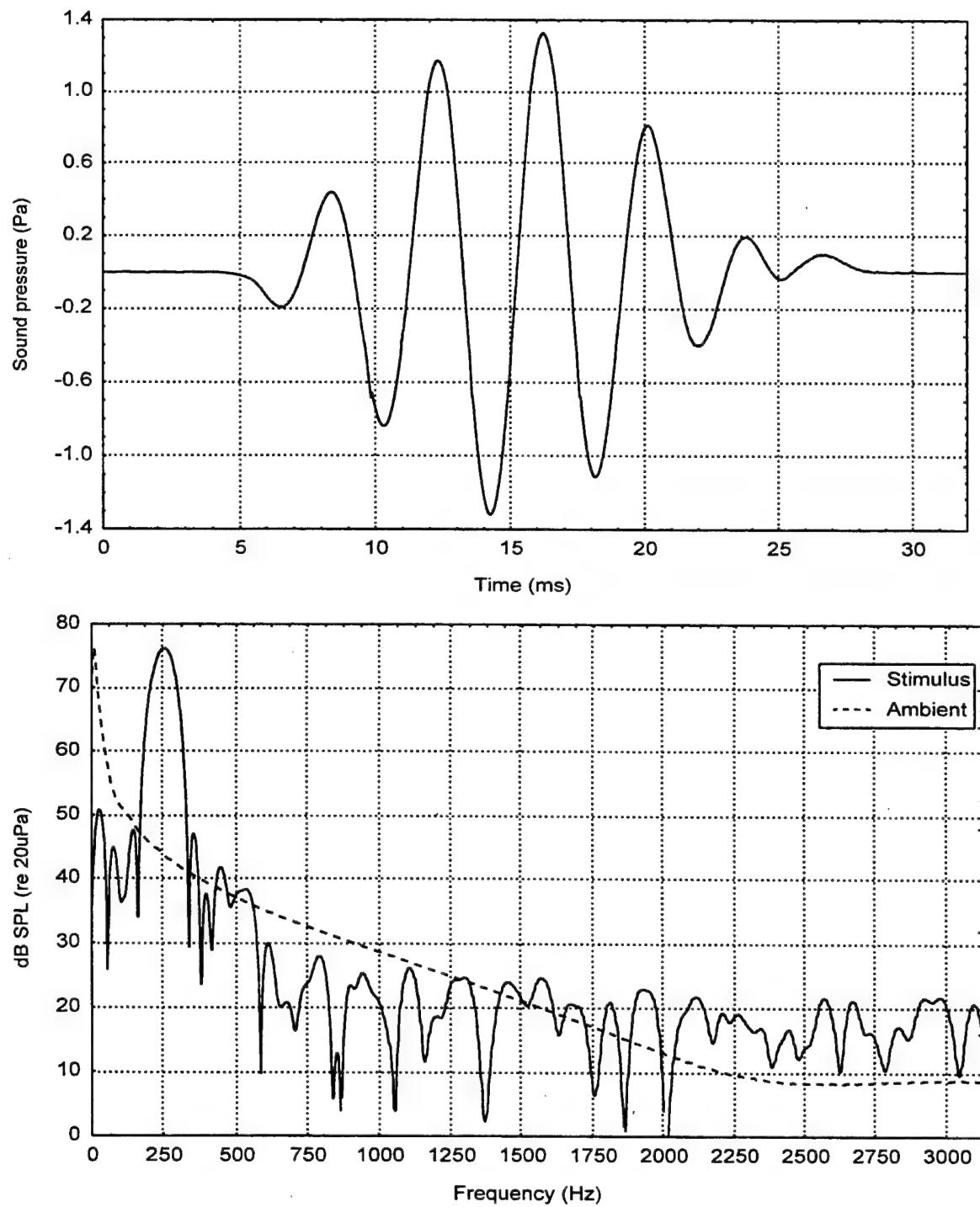


Figure 11. Waveform (top) and spectrum (bottom) of 250 Hz tone pip delivered to desert tortoises during ABR measurements. Spectral level of ambient was measured inside the earpiece and within the HSWRI sound isolation chamber.

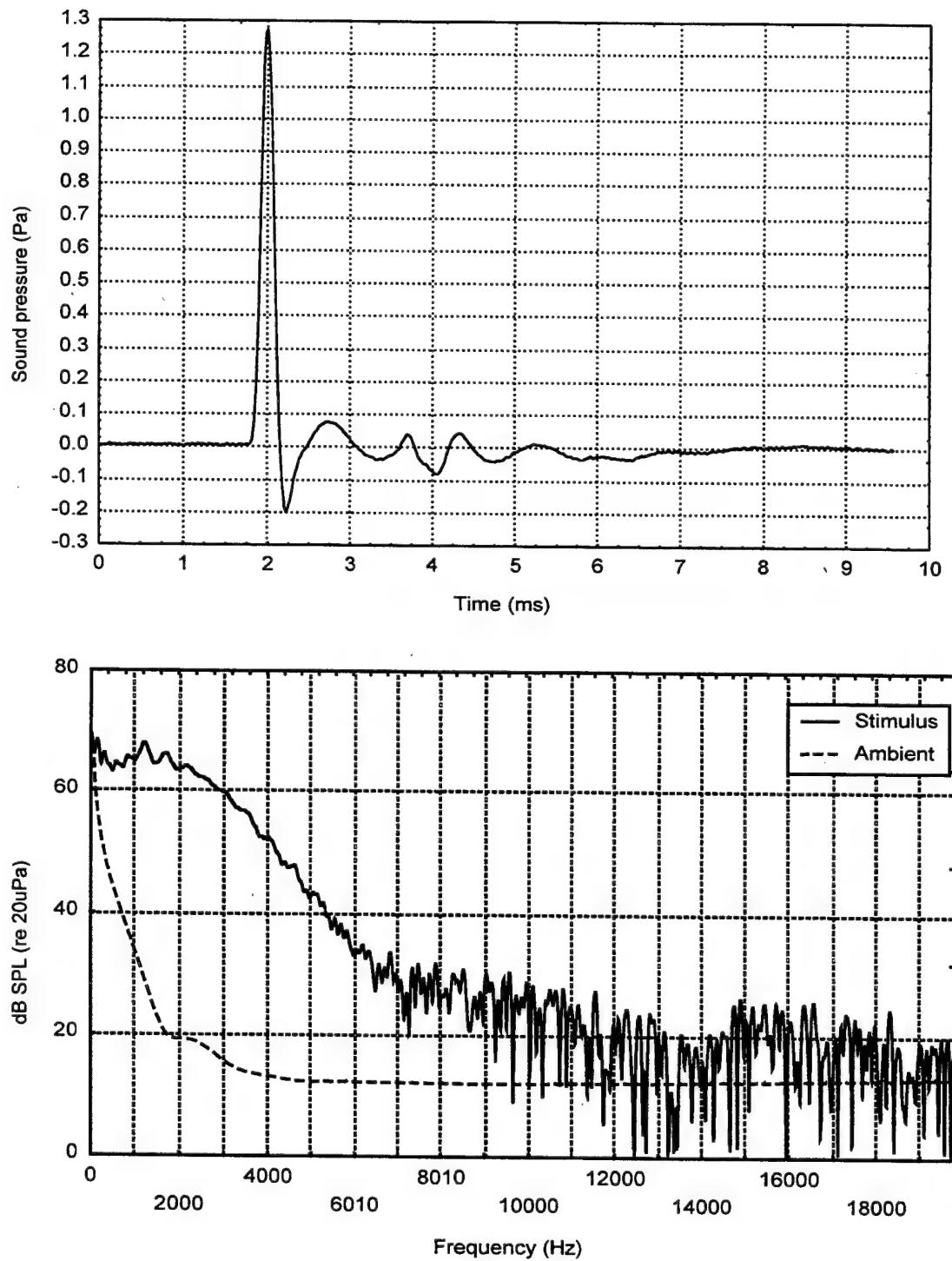


Figure 12. Waveform (top) and spectrum (bottom) of clicks delivered to desert tortoises during ABR measurements. Spectral level of ambient was measured inside the earpiece and within the HSWRI sound isolation chamber.

pips used in behavioral experiments might be more audible simply due to their greater duration.

This problem does not affect ABR stimulus presentations, however. To understand why, it is necessary to remember that the tone pips used to elicit ABRs are delivered many times per second. The relationship between RMS stimulus level and metrics most commonly reported in studies of noise are given in Table VII (event  $L_{eq}$  [equivalent to RMS SPL], SEL, and peak level). These values were calculated for stimulus levels from 55-95 dB (that is, the levels reported by the ABR instrument) and 250 Hz frequency. To produce this table, tone pips were recorded for 1 s, then weighted one of three ways (flat, A-weighting, C-weighting; in normal usage, they were not weighted). Stimulus presentation regimes is shown as well - continuous stimulus rate (no interval between pips; 43/s) vs. 29/s rate (used in the present experiments) vs. a single pip (1/s) vs. the steady-state portion of the tone pip (divested of its smooth onset and offset). The last was included because some investigators use short, unsmoothed tone pips that are more like clicks to elicit ABR waveforms.

Several important points become obvious upon examining this table. First, C-weighting and flat-weighting produce very similar levels for the narrowband 250 Hz signal; this similarity was found used in behavioral experiments would have similar perceived levels. However, single tone pips and stimuli presented at a slow rate would not. Studies of ABR amplitude have shown that intermediate presentation rates (10-30/s) yield the most detectable waveforms - they have perceived levels close to continuous tones, while reducing neurophysiological fatigue by allowing a recovery interval between successive stimuli.

ABR testing was conducted using a turnkey system, a portable Bio-logic model Traveler II computer. Stimuli at 250, 500, 1000, 2000, and 3000 Hz were presented using an Etymotic Research model ER-2 speaker. The ER-2 speaker is designed for insertion into the human auditory meatus as closely as possible to the tympanic membrane, to measure hearing level. Tortoises have no meatus; sound is transduced into the ear by the tympanum (Figure 2), a flat drumhead of skin on the side of the head. A short length of surgical tubing was glued around the tympanum with removable contact cement (Figure 13). The ER-2 insert was slipped into this sleeve, leaving a 1.43 cc space in front of the tympanum. This configuration allowed tortoise hearing level to be estimated accurately and, secondarily, reduced noise. All hearing tests were conducted in the IAC sound isolation chamber. The combination of IAC chamber and surgical tubing reduced outside noise below measurable levels at most of the frequencies of interest.

In order to collect ABRs, the subject animal had to be restrained and sedated to reduce artifacts

Table VII: Comparison of tone pip levels under different stimulus presentation regimes and using different weighting functions.

Stimulus Rate (#/sec)	Stimulus Level (dB)	Stimulus Type	Duration (sec)	Flat Weighted			A-weighted			C-weighted		
				RMS SPL*	SEL (dB)	Peak (dB)	RMS SPL*	SEL (dB)	Peak (dB)	RMS SPL*	SEL (dB)	Peak (dB)
43	95	fast rate	1.000	98.54	98.54	105.56	89.68	89.68	106.1	98.53	98.53	105.54
29.3	95	slow rate	1.000	96.76	96.76	105.5	87.87	87.87	96.56	96.76	96.76	105.44
-	95	single pip	0.036	96.62	82.12	105.45	87.71	73.22	96.56	96.61	82.12	105.43
-	95	steady state	0.008	105.18	80.01	107.28	105.15	79.98	107.3	105.18	80.01	107.26
43	85	fast rate	1.002	87.9	87.9	95.58	78.99	78.99	93.29	87.89	87.89	95.68
29.3	85	slow rate	1.000	86.09	86.09	94.91	77.18	77.18	85.91	86.08	86.08	94.87
-	85	single pip	0.036	85.87	71.45	94.64	76.96	62.54	85.9	85.86	71.44	94.75
-	85	steady state	0.008	91.18	70.35	94.69	82.26	61.43	85.89	91.17	70.34	94.7
43	75	fast rate	1.000	78.07	78.07	85.44	69.09	69.09	80.9	78.02	78.02	85.35
29.3	75	slow rate	1.000	76.28	76.28	85.25	67.37	67.37	76.92	76.25	76.25	84.97
-	75	single pip	0.035	76.1	61.56	85.26	67.16	52.62	75.95	76.07	61.53	84.93
-	75	steady state	0.008	81.25	60.42	84.45	72.32	51.49	75.97	81.24	60.41	84.82
43	65	fast rate	1.000	68.89	68.69	76.59	59.47	59.47	69.46	68.8	68.4	76.28
29.3	65	slow rate	1.000	66.8	66.8	76.14	57.67	57.67	66.77	66.59	66.59	75.48
-	65	single pip	0.035	66.71	52.12	75.59	57.69	43.09	66.41	66.64	52.05	75.46
-	65	steady state	0.008	71.71	50.92	74.32	62.68	41.9	66.44	71.66	50.87	75.22
43	55	fast rate	1.000	60.11	60.12	69.82	49.55	49.55	56.92	58.52	58.52	66.18
29.3	55	slow rate	1.000	58.87	58.86	68.67	47.83	47.83	56.8	56.83	56.83	66.43
-	55	single pip	0.032	57.37	42.47	63.32	48.03	33.13	56.37	57.08	42.18	64.35
-	55	steady state	0.008	62	41.25	63.9	52.83	32.08	56.66	61.89	41.14	65.26

due to muscle noise and electrode movement. Sedation was also needed to minimize discomfort to the animal while under restraint. Light sedation was used because deep anesthesia suppresses the ABR response somewhat. Tortoises were sedated lightly with an intramuscular injection of Diazepam using a dose of 0.8 mg/kg. The recommended dosage for reptiles is somewhat lower, but this was the minimum dosage needed to reduce activity in desert tortoises. After injection, all the tortoises appeared to be lightly sedated. They slept when not being handled, but wakened spontaneously throughout the experiment, when loud tones were presented, or when touched. The level of sedation varied among individuals - some individuals never slept, and one individual was always too mobile to make accurate measurements (LL 47). Although some individuals displayed the apnea typical of sleep, none exhibited unusual slowing of respiration. The dosage was not increased for restive individuals because the drug took effect slowly and was metabolized slowly, making adjustments in dosage difficult. At the dosage used, most individuals became fully sedated about 2 hours after injection and required 12-30 hours to regain full activity. For this reason, tortoises could not be sedated more often than once in 48 hours.

Once sedated, the tortoises were implanted with three subdermal platinum/iridium needle electrodes (Figure 13), one in the muscle of the upper foreleg (ground), one under the scutes between the eyes (active or vertex electrode), and one in the pad of muscle over the cochlea (reference or cochlear electrode). Electrode impedance was kept less than 10 kΩ and inter-electrode differences were kept less than around 5 kΩ. The sedated tortoises were kept at ambient temperatures of 28-32°C, with every effort made to keep the temperature constant (within 1°C) in the measurement chamber during the course of any given experiment. Temperature was recorded at the beginning and end of each series of ABR trials. Humidity was kept at 30-40% throughout.

After sedation, tortoises were restrained by tying them down on a mount by the gular horn and the rear points of the plastron, with the head and legs hanging free. The tortoise could move its head and legs, but could not get traction to move the shell. Its head was kept extended beyond the upper lip of the carapace with a soft plastic collar. In this position, the tortoise was comfortable enough to relax and sleep for most of each experiment. Respiration was timed periodically to insure that the tortoise was not too deeply sedated.

The levels of the stimuli produced by the Bio-logic system were calibrated by sealing a ACO 7013 1/2" microphone system in the position of the tortoises' tympanum and recording stimuli on a TEAC RD101T DAT recorder (system calibrated using a GenRad 1986 Omnical Calibrator generating 104.1 dB SPL at 1 kHz). Calibration parameters of the BioLogic system were cor-

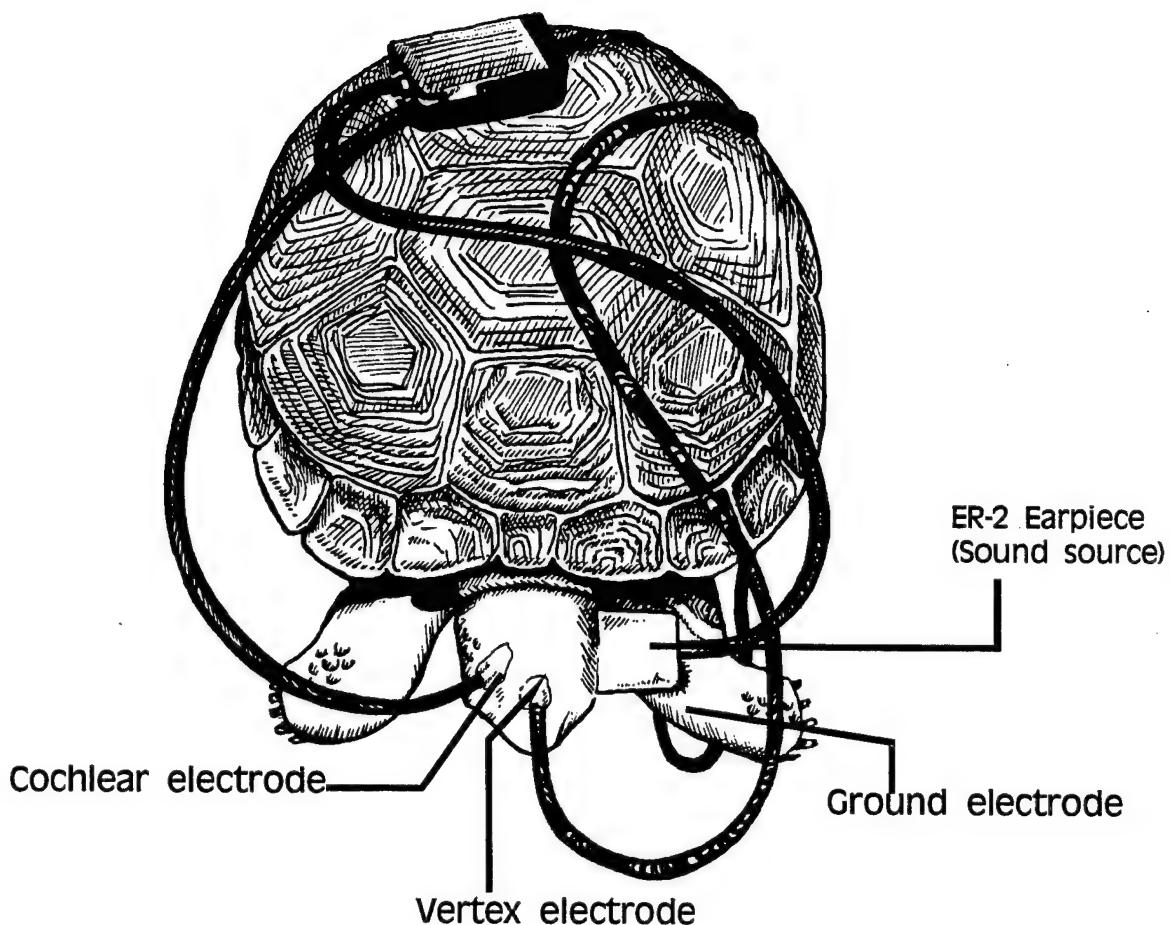


Figure 13. Position of electrodes and sound source (ER-2 or ER-3 earpiece) during auditory evoked response measurements (ABR, free field ABR, EFR).

rected to compensate for any deviations from expectation. This insured that the SPL of the stimuli actually delivered by the system were within 1-2 dB of expectation. At very low levels (below ~30 dB), it was nearly impossible to make accurate measurements of stimulus levels using the calibrated microphone system due to low signal to noise ratio. Under these conditions, the level reported by the instrument was presumed to be accurate.

Tone bursts were delivered initially at 250, 500, 1000, 2000, 3000, and 4000 Hz. There was little evidence of an ABR waveform above 1-2000 Hz at stimulus levels that could be delivered

without significant distortion (above ~90 dB, stimulus distortion was a significant factor). Most individuals were tested in the range from 250-1000 Hz.

After some initial experimentation, the optimal stimulus presentation rate was found to be 29/s. Although slower rates yielded somewhat greater ABR amplitude, they doubled the time necessary to complete a playback experiment. Rates faster than around 35/s yielded poor waveforms.

During experiments, click and tone burst level was decreased until no ABR could be detected, and then increased again to the initial level. Every effort was made to collect two replicates at each level-frequency combination. Replicates were collected to insure that waveforms were not the result of random electrical or electrophysiological noise - in order to be detectable, peaks in the ABR waveform had to occur at the expected latency, had to exceed the level of the background noise and had to appear in at least two replicates. Every effort was made to collect several estimates of click threshold in order to determine the error in successive threshold measurements.

The amplified output of the recording electrodes was filtered from 100-3000 Hz and sampled for 15-30 ms after the stimulus was presented. ABR waveforms were saved on disk for further analysis.

#### *Hearing below 200 Hz measured using low-frequency stimuli delivered in a free field*

The ER-2 ear insert speaker was not designed to produce low-frequency stimuli (<250 Hz). To determine sensitivity at the low end of the tortoises' hearing range, a number of different techniques were tested. First, tone pips were delivered to the tortoises using an ER-3 ear insert, which was designed to deliver low-frequency sounds. However, these tests did not yield clear AEP waveforms. Based on Patterson's (1966) results, it seemed likely that sensitivity to these frequencies would be better if delivered in a more natural manner, allowing the tortoises to receive the signal with both ears and through bone conduction (conduction through non-auditory tissues). Therefore, a few experiments were conducted with the test animal mounted next to a low frequency JBL4642 Subwoofer which had 2 JBL 2241 18" drivers in it capable of delivering 30 hz ( $\pm 3$ dB), 25hz (-10dB).

#### *Measurement of low-frequency responses using the envelope following response*

Sensitivity to low frequencies in some species, including humans, can be measured using the envelope following response, an AEP stimulated by a continuous, amplitude-modulated carrier. This technique has been used to measure low-frequency thresholds in a number of species (e.g., Lins *et al.* 1995, Dolphin 1995). The technique was tested on the desert tortoises. For the

purposes of these experiments, a continuous, amplitude modulated tone was delivered to the test tortoise (Figure 14) using the same setup as for other AEP measurements. AEP waveforms were collected continuously during presentation, with each sample onset phase-locked to the stimulus waveform. The test signal had a carrier frequency within the high end of the tortoises' hearing range (750-800 Hz), which was amplitude-modulated at the frequency of interest (50-250 Hz). The resulting AEP waveform was collected, and a spectrum was calculated. It was examined to determine whether there was a component at the target frequency. In theory, the amplitude of this component should be an indication of sensitivity at the modulation frequency, and threshold would therefore be measurable in a manner analogous to measurements of ABR peaks, using the least detectable waveform.

#### *Sensitivity to vibration*

Patterson's (1966) study showed that testudinates may feel rather than hear sounds at low frequencies. Sensitivity to vibration was measured in Patterson's study by coupling a vibration source to the head of the test animal. However, other parts of tortoises, particularly the feet and plastron, are more likely to receive ground-borne vibrations induced by aircraft, and many parts of the body might be sensitive to vibrations induced by airborne noise, including the shell, lungs, and legs. An effort was made to measure tactile sensitivity to sound using vibration-evoked potentials. A vibration source was coupled to ridges on the plastron, the points most likely to be in contact with the substrate.

The AEPs measured proved to be frequency following responses. Unfortunately, these AEP waveforms were indistinguishable from waveforms resulting from electrical crosstalk between the vibration source and receiving electrodes, making electrophysiological measurements impossible to distinguish from experimental artifact. Every effort was made to remove the crosstalk, but without success. Therefore, data collected using this protocol were rejected.

#### *Measurement of otoacoustic emissions*

Otoacoustic emissions (OAEs) are faint broad-band sounds produced by resonances in the outer hair cells of the inner ear after stimulation with a sound. They can be measured by placing a small microphone as close as possible to the cochlea; in the case of mammals, they are usually recorded from a microphone placed close to the tympanic membrane. In the desert tortoise and other reptiles, the closest access point is at the tympanum.

The preferred method for eliciting this response is the distortion-product otoacoustic emission (DPOAE) measurement. DPOAEs are elicited by delivering two tones (referred to as primaries)

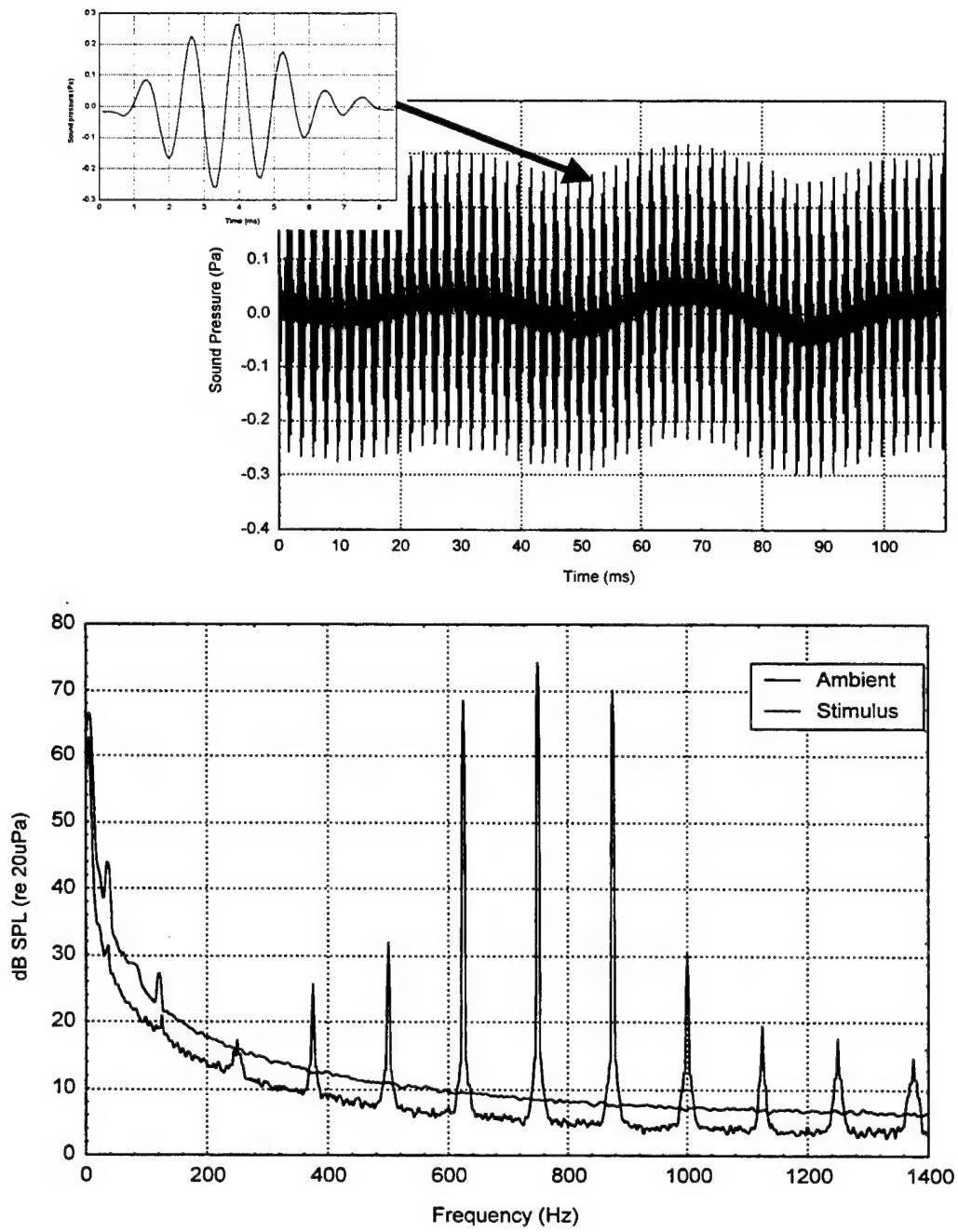


Figure 14. Waveform (top) and spectrum (bottom) of modulated waveform delivered to desert tortoises during EFR measurements. Insert shows an individual mode of the modulated signal. Example stimulus delivered at 70 dB RMS level. The carrier frequency was 750 Hz modulated at 125 Hz. The spectral level of ambient noise was measured inside the ER-3 earpiece and within the HSWRI IAC sound isolation chamber.

played through separate speakers. Because the OAEs are broadband, it is possible to measure them in the presence of the primary tones through appropriate filtering. The frequency of the stimulus was characterized by the geometric mean of the two tones (f1 and f2 [1])

$$(1) \text{ GM} = \sqrt{f1 \cdot f2}$$

and the ratio between them

$$(2) r = f1/f2$$

The frequency pairs delivered during DPOAE measurements had GM values from 250 to 2000 Hz. The DPOAE tests included the following steps:

1. A number of different frequency ratios (r) were tested to determine which produced the OAEs with the greatest amplitude (ratio test).
2. DPOAE amplitude was collected across the range of GM values and plotted against background noise levels (audiogram test).
3. DPOAE amplitude was measured as a function of the level of the primary tones that produced a given GM (input/output test). Primary tones were varied in 5 dB steps until amplitudes stopped changing (an estimate of threshold).
4. A third tone was added to the stimulus. This tone was varied around each of the primary frequencies until the DPOAE response was suppressed (suppression test). This test was designed to show that the signals received were actually otoacoustic emissions rather than artifacts.

All tests were conducted in the IAC sound chamber with sedated tortoises using the Otoscan software system. Stimuli were delivered using ER-2 speakers and returning acoustic signals were measured with an ER-10B microphone equipped with a size 15 ER 10-23 impedance tip. The whole assembly was mounted into the same rubber housing used for ABR measurements, which was glued around the test tortoise's tympanum. Three experiments were conducted using this technique (on LL42, LL47, and LL54).

### Noise-induced temporary threshold shift

Hearing of desert tortoises was measured before and after exposure to simulated sonic booms and aircraft noise. Tortoises were tested with clicks and tone pips at 250 and 1000 Hz before and after exposure to noise, *i.e.* at the best frequency and at the high frequency end of the tortoises' hearing range. Test conditions were similar to the initial hearing tests. However, the trials lasted somewhat longer and were conducted at slightly higher temperatures (30-33°C *vs.* 28-30°C) due to prevailing summer temperatures when these experiments were conducted. Click-evoked thresholds were measured at the beginning, immediately after exposure, and at the end of the post-exposure period, to document pre-exposure threshold, threshold shift induced by exposure, and recovery.

Three types of exposure experiments were conducted. Sub-sonic noise exposures delivered the 44 minute subsonic aircraft noise tape. The maximum overflight level exceeded 114 dB CSEL (Table II). The overflights exceeded background noise levels in the sound isolation chamber by 40 dB (Figure 15).

Experiments simulating worst-case carpet booms delivered 2 6-psf booms. Experiments simulating focused booms exposed tortoises to 10 6-psf booms (Table III).

During these measurements, tortoises were equipped with an accelerometer mounted on the lowermost ridge of the plastron between the front and rear legs to document the vibrations induced in the shell during exposure. These measurements were designed to determine whether the induced vibrations from the simulated noise were greater or less than the tortoises' vibration threshold.

### Measurement of Behavioral Responses

#### Behavioral responses to playback of subsonic aircraft noise

Originally, playback experiments were to have been conducted by comparing a day of baseline measurements with a day of simulated exposures. Initial baseline measurements made it clear that tortoises required periods of several weeks to become acclimated enough to a new space to make behavior on successive days comparable. Tortoises altered feeding and activity patterns unpredictably in the first few days after a change in habitat. As described by Ruby *et al.* (1994b), tortoises encountering barriers spend many hours trying to find a way out of their pens, a behavior that persists from hours to days. In the tortoise habitat at HSWRI, this behavior never disappeared completely, but it became uncommon after about 2 weeks in the home pen. It seemed unlikely that completely normal movements would be encountered in the IAC test cham-

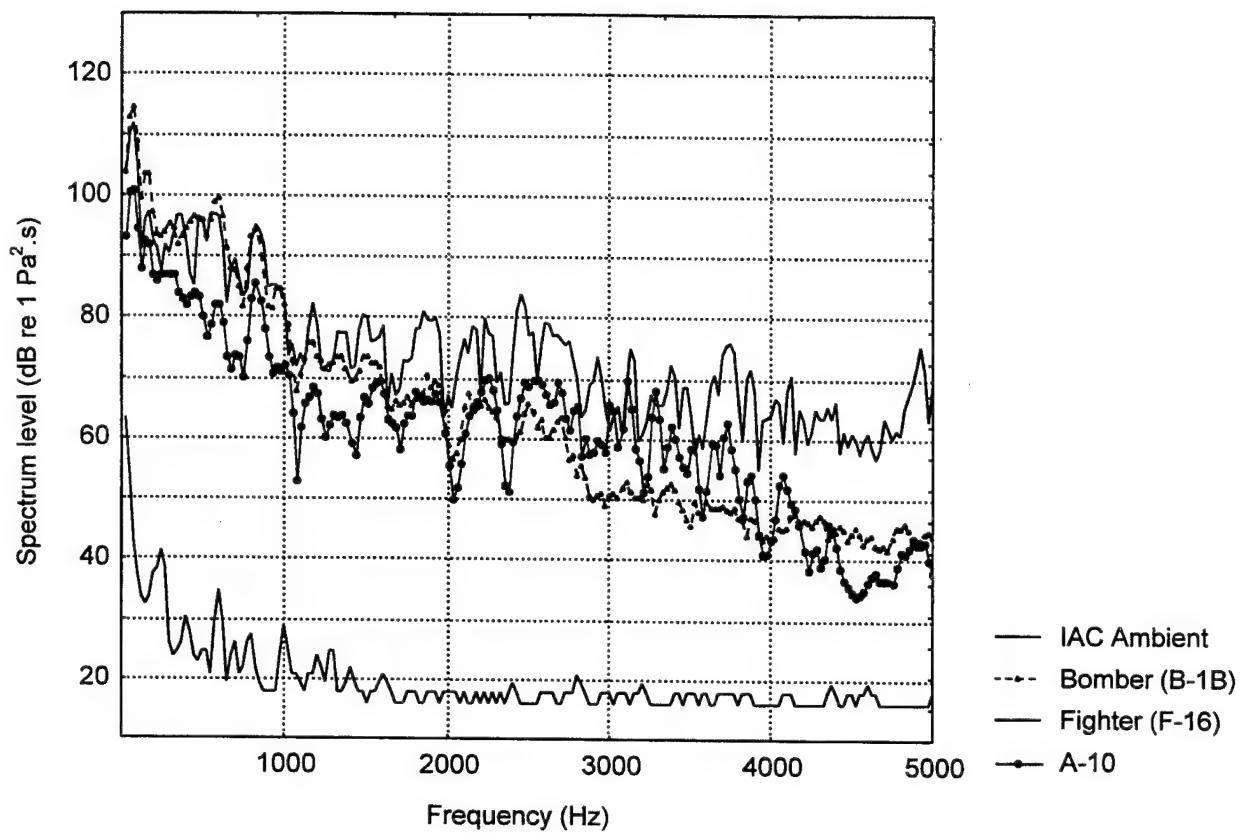


Figure 15. Spectra of overflight stimuli delivered to desert tortoises during sub-sonic noise playback and hearing experiments.

ber without lengthy acclimation, but it was impracticable to move the tortoises one at a time into the IAC chamber for several weeks before exposure. Therefore, the tortoises were introduced the night before exposure experiments and their behavior was monitored throughout the day of the test. Differences in activity patterns between the test chambers and the tortoise habitat were documented.

Table VIII summarizes the subsonic aircraft noise experiments. Responses of desert tortoises to playback of subsonic aircraft noise were documented in the sound isolation chamber using two procedures. Eight untethered tortoises received exposures in the 0.61x1.83m (2x6 ft) pen mounted inside the sound isolation chamber. They were given their own bedding, burrow and food tray. The three remaining tortoises were fitted with long leads to the heart rate monitoring

Table VIII. Experiments to measure behavior and heart rate response to subsonic aircraft noise.

Date	Tortoise	Total Duration of Observation	Time of Exposures	Playback Condition
7/25	LL47	14:37 on 7/24 to 14:00 on 7/25	None	Baseline
7/26	LL47	15:22 on 7/25 to 23:49 on 7/26	None	Baseline
8/4	LL54	07:29 on 8/4 to 01:15 on 8/5	None	Baseline
8/5	LL54	01:18 on 8/5 to 22:50 on 8/6	07:50 11:00 17:50	Behavior monitoring
8/6	LL6	23:34 on 8/6 to 01:15 on 8/7	07:48 11:00 17:48	Behavior monitoring
8/7	LL94	01:48 on 8/7 to 21:35 on 8/7	09:12 11:30 16:51	Behavior monitoring
8/13	LL47	22:20 on 8/22 to 22:32 on 8/23	08:21 11:00 16:58	Behavior monitoring
8/17	LL42	21:51 on 8/16 to 22:01 on 8/17	08:32 11:04 18:19	Behavior monitoring
8/18	LL32	23:15 on 8/17 to 22:01 on 8/18	08:28 11:01 18:08	Behavior monitoring
8/19	LL1522	22:33 on 8/18 to 22:20 on 8/19	08:42 11:33 16:49	Behavior monitoring
8/21	LL4	22:43 on 8/20 to 22:50 on 8/21	09:49 12:23 17:43	Behavior monitoring
9/23	LL1522	18:18 on 9/22 to 17:12 on 9/23	08:30:00 11:03:00 15:21:00	Behavior and HR monitoring;
9/24	LL1410	18:31 on 9/23 to 17:00 on 9/24	08:30:00 11:10:50 15:06:00	Behavior and HR monitoring;
9/25	LL1	17:47 on 9/24 to 17:02 on 9/25	08:32:00 11:37:00 15:02:00	Behavior and HR monitoring;

Table VIII (cont'd) Experiments to measure behavior and heart rate response to subsonic aircraft noise.

Date	Tortoise	Total Duration of Observation	Time of Exposures	Playback Condition
10/10	LL32	7:36 to 18:00	9:30:00 12:00:00 15:58:00	Behavior and HR monitoring;
10/11	LL4	7:50 to 16:11	9:07:49 11:30:30 15:28:30	Behavior and HR monitoring;
10/12	LL6	8:24 to 17:26	9:15:00 11:43:45 15:40:20	Behavior and HR monitoring;
10/13	LL1355	8:54 to 16:18	9:28:30 11:29:00 14:58:30	Behavior and HR monitoring;

system, then released into the same chamber. These tortoises did not have access to a burrow, but were kept on their own bedding with their own food dish.

The tortoises were introduced into the test chamber the night before the experiment. This allowed them to acclimate to the chamber and to begin the trial the next morning in a resting state. The chamber was not opened during the experiment except to feed the test tortoise between 0930 and 1030 hrs (the usual feeding time). A Canon L1 camera connected to a time-lapse video recorder (Panasonic AG-6024) was mounted overhead to allow a good view of every part of the pen, collecting video of the entire experiment at 2.5 frames/sec. At this resolution, video analysts could detect all but the most subtle behaviors of the tortoise, including head withdrawals, dropping to the ground, leg withdrawals, and other defensive behaviors. Only small movements of the head and feet were missed, *e.g.*, gaping. The video recorded behaviors during the daylight hours, from 0600 to 2200 hrs.

Free-ranging tortoises were exposed to jet aircraft noise once before being fed (~0830 hrs), once after being fed (~1100 hrs) and once after they had quieted for the evening (~1700 hrs). Tortoises attached to the heart-rate monitor received their first two exposures on the same schedule,

but received their last exposure at ~1500 hrs for logistic reasons. The chamber filtered out noise of humans talking and working in the laboratory space at HSWRI very efficiently, but it did not isolate the tortoises from low frequency rumble emitted by aircraft from nearby Lindbergh Field Airport and heavy trucks on nearby city streets. These rumbles were clearly audible to the acclimated human ear when the chamber was shut up and exceeded the threshold on the community noise monitor from time to time on the C-weighted setting.

An ethogram for the behavioral analysis is given in Appendix B. Four types of data were collected: (1) counts of behavioral events before, during, and after exposure, (2) time spent in different activity states (activity budgets), (3) latencies to some events (particularly defensive behaviors) after each simulated noise exposure and (4) incidence of changes in behavior immediately before and after exposure. Events were single behaviors, e.g., head retraction, whereas states were defined by sequences of behaviors (e.g., climbing, which consisted of periods of scrambling against the walls of the test pen interspersed with bouts of resting, rooting in the bedding, or moving to a new location).

#### *Behavioral responses to playback of simulated sonic booms*

Tortoises were first exposed to simulated sonic booms without the heart-rate monitoring tether. These exposures were conducted during a one-day stay in the INTF; six additional experiments were conducted with the heart-rate leads attached (four of the tortoises in these experiments had already heard sonic booms once; Table IX). The tortoises were placed into the INTF chamber an hour before the first exposure and were removed at least an hour after the last. A camera was mounted in the side of the chamber, but space constraints left a blind spot just underneath the camera post. This blind spot was filled with a cardboard barricade to exclude the tortoises. Tortoises occasionally got out of the field of view by burrowing under the barricade or hiding behind their burrow, but for the most part they were visible throughout the experiments. Tortoises were introduced into the chamber at 0800-0830 with the day's ration of food and were removed at 2200-2230 hrs, for a total of 14 hours of observations.

During the untethered trials, tortoises were exposed to two series of 10 sonic booms, once in the morning (1030-1140 hrs) and once in the evening (1900-2040 hrs), maximizing the chances of exposing them once when active and once when quiescent. The exposure series (Table IV) was designed to show changes in responses during successive exposures and the effect of a change in sonic boom level. The 10 minute exposure interval proved to be too short to detect changes in activity from individual exposures, as the tortoises often did not exhibit any short-term startle or change in activity. Several other exposure regimes were tested thereafter. First, three tortoises

Table IX. Experiments to measure behavior and heart rate response to simulated sonic booms.

Date	Tortoise	Total Duration of Observation	Time of Exposures	Playback Condition
8/24	LL1	09:25 to 22:07	10:30 19:00	Script 1; Behavior monitoring
8/25	LL1410	09:36 to 22:38	10:30 19:00	Script 1; Behavior monitoring
8/26	LL93	08:52 to 23:55	10:30 19:00	Script 1; Behavior monitoring
8/28	LL1	08:30 to 22:00	10:30 19:00	Script 1; Behavior monitoring
8/31	LL6	10:05 to 23:22	10:30 19:00	Script 1; Behavior monitoring
9/1	LL54	08:47 to 22:20	10:30 19:00	Script 1; Behavior monitoring
9/2	LL32	08:29 to 22:04	14:34	2 booms in quick succession; Behavior monitoring
9/3	LL32	08:22 to 22:30	10:30 19:00	Script 1; Behavior monitoring
9/7	LL42	10:03 to 02:22 on 9/8	10:30 19:00	Script 1; Behavior monitoring
9/9	LL47	09:17 to 22:04	10:30 19:00	Script 1; Behavior monitoring
9/10	LL1522	08:29 to 22:15	10:30 19:00	Script 1; Behavior monitoring
9/11	LL4	08:30 to 22:31	10:30 19:00	Script 1; Behavior monitoring
9/27	LL32	08:30 to 15:03	10:30 12:40	Script 2; HR monitoring
9/28	LL42	9:00 to 15:44	10:30 12:40	Script 2; HR monitoring
9/29	LL1355	08:48 to 15:37	10:30 12:40	Script 2; HR monitoring
10/16	LL1	07:06 to 16:55	8:30 (0.25 psf) 10:30 (1.00 psf) 12:30 (6.00 psf) 15:00 (0.25 psf)	Script 3; Behavior and HR monitoring
10/17	LL94	07:15 to 17:30	8:30 (0.25 psf) 10:30 (1.00 psf) 12:30 (6.00 psf) 15:00 (0.25 psf)	Script 3; Behavior and HR monitoring
10/18	LL1410	06:23 to 17:00	8:30 (6.00 psf) 10:30 (1.00 psf) 12:30 (0.25 psf) 15:00 (1.00 psf)	Script 4; Behavior and HR monitoring

were exposed to a block of booms (Table V) in the morning, to look at overall effects on activity, and to a wide range of levels in the evening. These tortoises were attached to the heart rate monitoring tether. A second set of tethered experiments exposed three tortoises to single booms at long intervals. During this set of tethered trials (Table IX), 4 sonic booms were delivered, separated by 2-4 hours. The levels of the sonic booms were varied to determine (if possible) the relationship between heart rate and exposure (0.25, 1.0, and 6.0 psf). The order of presentation was varied to eliminate order bias.

### **Measurement of the Relationship Between Metabolic Rate and Heart Rate**

Metabolic rate and heart rate were measured simultaneously with the tortoises under three experimental conditions: (1) resting (2) exercising on a treadmill and (3) exercising on a treadmill with an indwelling catheter for measuring blood lactate concentration. Lactate measurements were included as a means of determining whether the test tortoise was exercising at its maximum aerobic level ( $VO_2$  max). All three types of experiments were conducted in the tortoise habitat, with the ambient temperature held at 30°C. Body temperature was recorded before and after each exercise period using a thermocouple inserted 5 cm into the cloaca.

#### **Resting metabolic rate**

Tortoises were not fed (fasted) for three days before experimental sessions. Resting metabolic rate measurements were determined for quiescent animals at night in total darkness. The animals were placed in a box (45 x 15 cm) containing bedding material and a burrow from their own enclosures. Each recording period lasted for approximately 2 hours. If the animals were active at all during that period, the measurements were repeated to insure a true resting value.

Tortoises were restrained for instrument placement by putting them on a 35 cm pedestal and hooking two elastic bands over the gular horn and rear points of the plastron. The top of the pedestal was a board shaped to approximate shape of the plastron. While restrained in this manner, tortoises were able to wave the head and legs freely, but could not obtain purchase to turn themselves. They relaxed and even fell asleep when restrained in this manner.

#### **Oxygen consumption**

Oxygen consumption was measured using a flow-through system (Figure 16). In such a system air is drawn through a mask adhered to the tortoise's face. Oxygen content of the outflow air is monitored by an oxygen analyser after first passing it through a Drierite/Sodasorb column (to

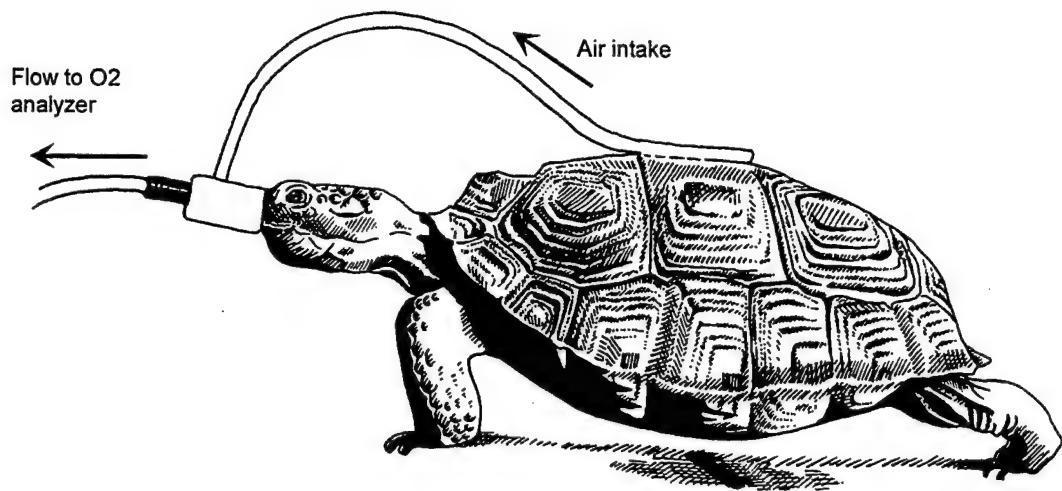


Figure 16. Desert tortoise equipped for measurements of oxygen consumption.

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remove water and carbon dioxide). The resulting partial pressure of oxygen ( $PO_2$ ) is compared to the known  $PO_2$  of the inspired air to calculate oxygen consumed. Mixing of exhaled and inspired air (i.e. dilution of the expirant) is impossible with this system because the high rate of flow prevents backflushing. The great advantages of such a system are: (1) the tortoise can be allowed to move about freely within the range of its tether and (2) there is no opportunity for  $CO_2$  stress.

Each tortoise was fitted with a mask made of polyvinyl tubing with the end flared to fit snugly over the nostrils (Figure 16). It was not necessary to cover the mouth, as the tortoises did not breathe through their mouths while exercising. The top side of the mask had a small (2.5 cm long) piece of tubing inserted through the sidewall to act as an air intake. Inserted into the end of the mask was another polyvinyl tube (1/8" I.D. x 1/4" O.D. x 1/16" wall, 175 cm long) attached to a Drierite/Sodasorb column, and from there to an Ametek S3-A oxygen analyzer. To attach the mask, the tubing was initially set in place with Vet-Bond, a cyanoacrylate adhesive made especially for tissue bonding (most commercial cyanoacrylate adhesives give off detrimental fumes during curing and should not be used under the conditions described here.) After attachment, the mask was sealed to the tortoise's face with Devcon 5-minute epoxy. This mask did not appear to adversely effect the tortoises; they fed and moved about normally in their pens with the mask in place between measurements. Upon completion of the experiments, the mask

and epoxy peeled off cleanly, with no harm to the tortoise.

Rate of air flow was maintained at 220 ml/min with an Ametek R-1 Flow Controller. The response time between exhalation and subsequent detection by the O<sub>2</sub> analyzer was very rapid (6-10 seconds).

#### Metabolic rate during exercise

Tortoises were trained to run on a treadmill. Because the treadmill (Accusmart Proform 930) was originally designed for human use and moved too rapidly for the tortoises, the speed controller (potentiometer) was replaced and the instrument was recalibrated for belt speeds as slow as 0.02 m/s with a precision of 0.001 m/sec. To restrict the tortoises to the treadmill and to screen them from distractions while walking, an open-top box was built (100 x 50 x 35 cm) which was elevated 3 cm over the belt, enclosing the animal, yet allowing the belt to travel freely. The box walls were painted flat black and a small window was cut out of the front end to provide the tortoises with an obvious exit. The treadmill was kept at 0° of inclination (level) for all experiments.

Tortoises were initially trained (once or twice per animal) to walk without a mask or heart rate leads. During the actual experiments, a tray of food was placed in the window at the front of the cage to motivate the animals to walk. Although they were not allowed to eat from the tray, they continued walking towards the opening steadily, for up to 2 hours in some cases.

#### Heart rate

Electrocardiographic (ECG) signals were recorded continuously during resting and exercising experiments. Electrodes were made by soldering 1-cm diameter pure silver disks to 1-mm diameter silver wire approximately 2.5 cm long. They were attached to the tortoise by drilling a small diameter (1 cm) depression (about 3 mm deep) into the carapacial bone with a small rotary tool (Dremmel drill). Three electrodes were needed to monitor ECGs. Placements were (1) between the nuchal and first lateral scute, (2) between the fourth vertebral and fourth lateral scutes, and (3) between the fifth and sixth marginal scutes (ground). Such placement maximized the distance across the heart, hence electrophysiological potential, giving maximum signal with minimum noise artifact, even during exercise. The electrodes were coated with conductive cream and placed in the depressions. They were held in place with a cap of quick-setting epoxy. These electrodes remained patent for months after placement, although they were eventually expelled by growth of the carapace from underneath. To further enhance signal detection, the polarity of the electrodes was reversed, so that the QRS waveform appeared in the ECG trace as a downward voltage spike. This step was taken because most other electrophysiological noise

appeared as upward (positive) spikes. Low-resistance 3-m long speaker cables (12 gauge Monster-type cables) were attached to the silver wire of the electrodes and suspended centrally above the treadmill so that the animal could walk unconstrained.

#### Data acquisition

Heart rate and metabolic rate data were acquired automatically with a BIOPAC Systems Inc. MP100 and AcqKnowledge™ software running on a Pentium PC. This system simultaneously monitored inputs from the treadmill, O<sub>2</sub> analyser, and an ECG amplifier. BIOPAC's Acknowledge™ 3.0 software allowed real-time calculation of VO<sub>2</sub>, treadmill speed and heart rate. With the input of barometric pressure, temperature, relative humidity and air flow, and tortoise weight, Acknowledge calculated VO<sub>2</sub> at Standard Temperature and Pressure Dry (STPD). All variables were sampled and the calculated measurements were plotted continuously to the computer's screen. VO<sub>2</sub> was calculated using the following equations (Withers 1977):

$$(1) \text{ STP Flow} = \text{Air Flow} * (273^{\circ}\text{K}) * (\text{Barometric Pressure} - \text{Relative Humidity}/100)/760$$

$$(2) \text{ VO}_2 = \text{STP Flow} * (0.2094 - \text{O}_2) * 1000/(1-\text{O}_2) * \text{kg}$$

#### Blood lactate levels

Blood lactate levels were measured to determine whether the tortoises were exercising within their aerobic limits during the metabolic rate experiments. An indwelling catheter had to be placed to enable the collection of serial blood samples on exercising tortoises without disturbing the walking animals. A feline catheter (Sovereign 22G needle 25G x 1.25") was introduced percutaneously under sterile conditions into the jugular vein or carotid artery. As the vessels were often difficult to locate, a new technique was developed to make the vessel visible before placing the needle. The tortoise was restrained on the pedestal with its neck extended. Room lights were darkened and the vessels were back-lit with the focused beam of a microscope light. The catheter needle was then placed into the clearly-visible vessel. Once the catheter was in, the neck was wrapped with a stretch bandage and a small amount of vet bond was applied to the catheter to keep it in place. A modified extension tube set was attached to the catheter and utilized for blood sampling. After insertion, the catheter was flushed periodically with saline solution to maintain patency (0.9% Sodium Chloride Injectable).

The tortoises were allowed to recover quietly for two hours before taking a baseline blood sample to allow lactic acid built up during handling to metabolize. Tortoises were then exercised on the treadmill and 0.2 ml samples of blood were collected at 10 min intervals (not

exceeding 10 samples/animal) while walking. Heart rate and metabolic rate were measured simultaneously. After exercising, the animals were allowed to recover before the catheters were removed. Once the catheters were out, tortoises were monitored for several hours in their enclosures to insure early detection of any vascular damage.

All blood samples were injected into a 1 ml heparinized tuberculin syringe after withdrawal of 1 ml to clear the dead space of the sample tubing. Lactate and glucose analyses were performed using a YSI 2300 Lactate/Glucose analyzer. A cell lysing kit was added to the reagent buffer to lyse the cells before analysis. Each sample was analyzed twice, and a third time if the values did not agree with one another.

*Analysis of heart rate and metabolic data.*

All data were analyzed using the Statistica™ statistics software. Metabolic and heart rates were averaged for each tortoise and plotted. Correlation between heart rate and metabolic rate was determined for individual tortoises as well as collectively for all tortoises using nonlinear estimation. Accuracy of the resulting model was tested by plotting the residuals against expected normal probability.

Heart rate data were quantified and averaged using the Acknowledge software for the exposure experiments. Average heart was calculated in 5 minute increments for 60 minutes prior to, during and post overflight or sonic boom exposures. Independent 't' tests were used for pair-wise comparisons of the pre, during and post periods for individual tortoises. Dependent 't' tests were used for pair-wise comparisons of all tortoises combined. Differences were considered significant at  $p<0.05$ .

## RESULTS

### Characteristics of Experimental Tortoises

All the test tortoises were in good condition at the time of capture based on a visual examination. A medical examination, blood tests and fecal samples within a few days of capture revealed that one animal was mildly symptomatic for URTD (LL1410, it had nasal discharge and an elevated white-cell count), four were anemic (LL1, LL6, LL47, and LL94), nine were found to have internal parasites (strongyloids, strongyloid larvae, ascarids, and oxyurids), five were found to have large numbers of bacteria, mostly gramm-positive rods and cocci, and one was later found to be gravid (LL52), although the eggs were not revealed in x-rays taken at entry. None had significant lung congestion. All were rid of parasites and fully hydrated within the first month of capture.

Eight females and six males were collected (Table X). The tortoises ranged in size from a 1.2 kg, female to a 3.6 kg male. The females were significantly shorter and lighter than the males even after their weight gain in captivity. Four of the tortoises had the shell wear typical of older animals, but shell length and wear does not provide an accurate estimate of age. Midline carapace length was used as a rough indicator of age in analyses. All but the largest male tortoise (LL1410) gained weight substantially during the course of the experiments, with an average weight gain of 20%. Some of this gain was the result of hydration, but not all of it; several of the tortoises gained over 1 kg.

The animals varied in responsiveness to humans and activeness. LL93 was often inactive and was rarely responsive to humans, although he gained 26% over the course of the study and was highly active in bouts when humans were not present. LL94 and LL32 were the most responsive to human activity in their environment, approaching keepers whenever they were present in the habitat. They also climbed and dug actively in their pens. LL1410 and LL4 were large animals with worn shells that responded only slowly to human presence, although they were alert and active when appropriately motivated (e.g., by food).

Table X. Sex, weight, length, and condition of subject tortoises

ID/SEX	Weight at Entry	Ending Weight	Weight gain (% of starting weight)	Carapace length (cm)	Condition at entry
LL1 - M	2.536	3.188	20.4	31.38	High white count
LL4 - M	3.357	3.770	11.0	26.67	
LL6 - M	2.165	2.772	21.9	28.89	Anemic
LL15 - F	1.152	1.712	11.4	25.72	
LL32 - F	1.581	2.329	32.1	20.96	
LL42 - M	2.545	3.574	28.8	32.38	
LL47 - F	2.096	2.808	25.3	29.53	Anemic
LL52 - F	2.183	2.52 (7/30)	13.4	22.22	
LL54 - F	2.087	2.291	8.9	28.58	
LL93 - M	1.425	1.928	26.1	25.08	Low heterophyll/lymphocite ratio; often inactive; unresponsive to humans
LL94 - F	1.620	2.135	24.1	27.30	Anemic
LL1355 - F	1.481	2.120	30.2	19.68	Low heterophyll/lymphocite ratio
LL1410 - M	3.604	3.708	2.8	26.67	mild URTD
LL1522 - F	1.655	2.253	26.5	21.59	

### Sound Transmission Into Burrows

The test burrow attenuated noise by 15-20 dB at frequencies above the hearing range of the desert tortoise (> 1500 Hz; Figure 17). In their estimated best range (200-800 Hz), it attenuated noise by ~10 dB. At very low frequencies (~100 Hz), there was a peak that exceeded the level of the input signal by 10 dB. This was probably the result of burrow resonance. The burrow therefore afforded only moderate protection from noise exposure and may actually have increased the perceptibility of low frequency noise components.

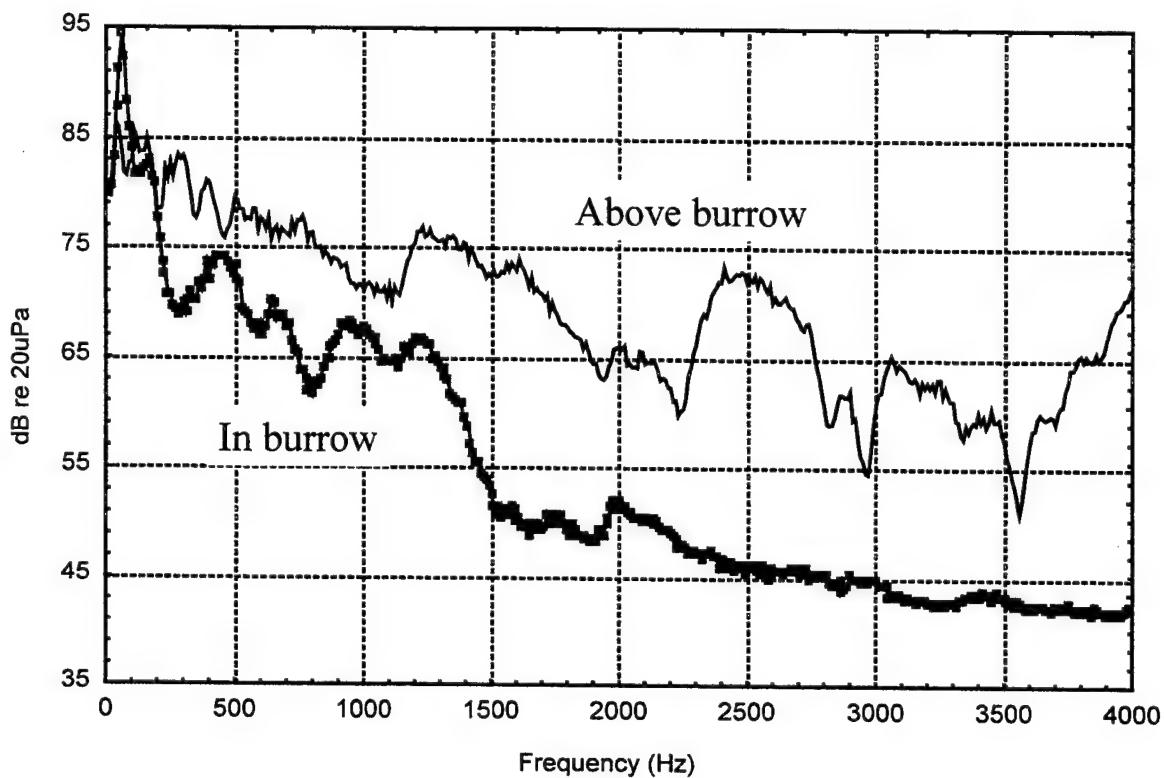


Figure 17. Spectra recorded 82 cm down a tortoise burrow and 1.2 m above ground. Short bursts of white noise were delivered to generate the comparison.

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#### Measurement of Hearing and Vibration Sensitivity

Measurements of tortoise hearing are summarized in Table XI. All 14 tortoises were tested at least once. LL52 died during the series of experiments ; only one measurement of hearing sensitivity was completed on this animal. Other individuals were only used 2-3 times due to (1) poor health (LL1355, LL1522), (2) poor response to sedation (LL47), or (3) difficulty in identifying ABR waveforms (LL1410).

#### Hearing above 200 Hz

ABR waveforms were detected reliably in response to click stimuli. These waveforms were highly repeatable, at least at high stimulus levels (Figure 18), and were similar in structure from individual to individual (Figure 19). They were designated as P1-P7 for the purposes of this report (no formal designation has been established for reptiles). Four peaks appeared to be characteristic of the tortoise ABR; in some cases, P3 and P4 tended to merge together in some

Table XI. Summary of experiments to measure auditory evoked potentials and otoacoustic emissions of desert tortoises.

Tortoise	Number Experiments *	Type of Measurement					ABR pre- and post- exposure 2 simulated booms	ABR pre- and post- exposure 10 simulated booms
		ABR earpiece	ABR free-field	Vibration -evoked potential	Modulation rate transfer function	Otoacoustic Emissions	ABR pre- and post- exposure sub-sonic noise	
LL1	4	1	1		1		1	
LL4	4	1	1	1			1	
LL6	5	2	1	2	1		1	
LL15	4	3		1				1
LL32	5	2	1			1		1
LL42	4	1				1		
LL47	3	1				1		1
LL52	1	1						
LL54	6	2		1	1		1	
LL93	8	5	1	1	2			2
LL94	7	3	1	2	1		1	1
LL1355	2		1					1
LL1410	3	1					1	1
LL1522	2			1				
<b>TOTAL</b>	<b>58</b>	<b>23</b>	<b>5</b>	<b>8</b>	<b>7</b>	<b>3</b>	<b>6</b>	<b>10</b>

\* More than one measurement could be conducted during the same experiment (i.e., period during which the tortoise was sedated). Therefore, the total number of experiments is often less than the sum of all measurements conducted.

† This tortoise received 10 booms rather than 2 in an experiment as a result of experimenter error.

‡ One ABR measurement on this tortoise was conducted with the animal at low temperature (24-29° C).

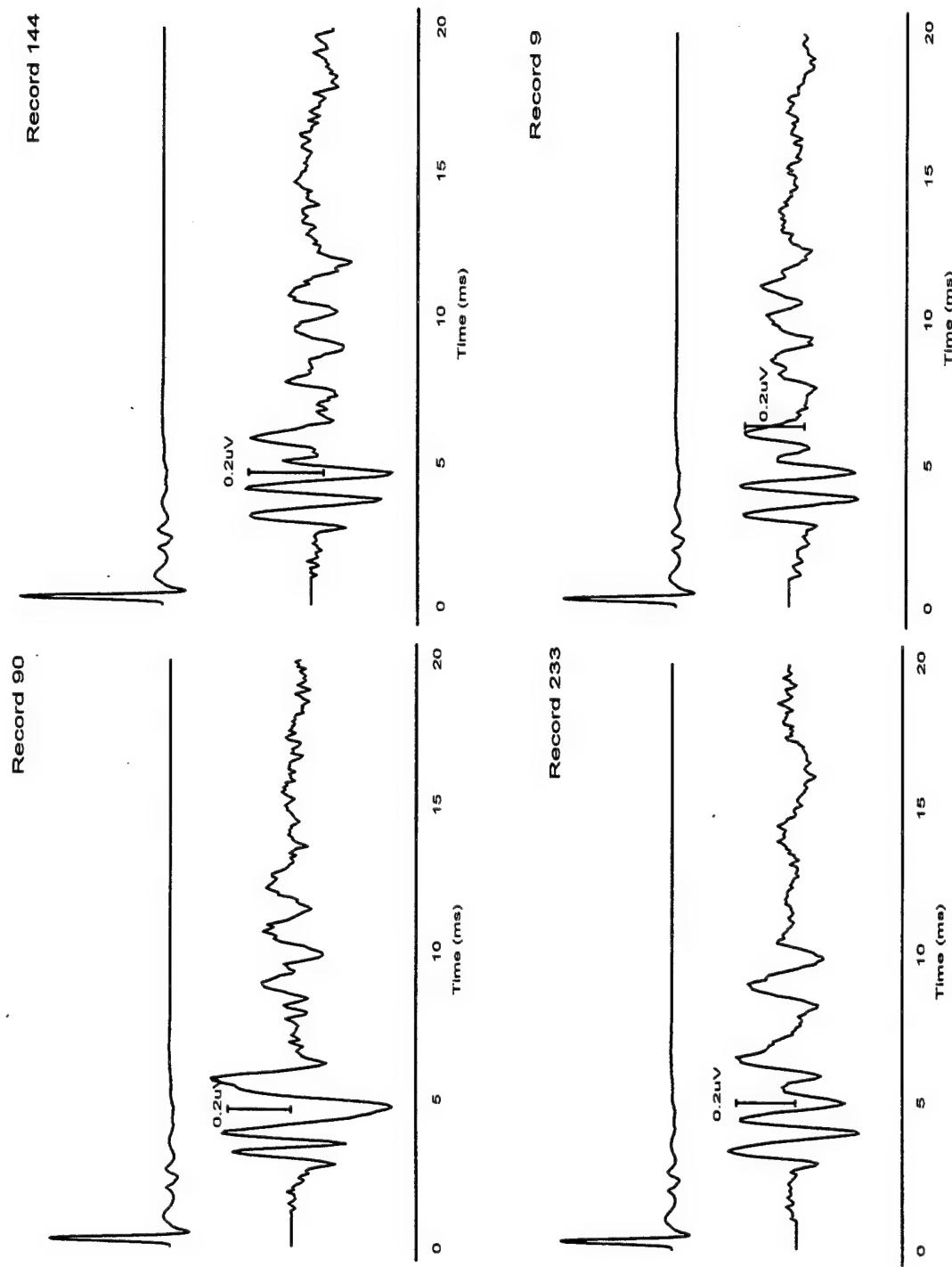


Figure 18. Four separate ABR waveform measurements elicited from tortoise LI.94 by the click stimulus.

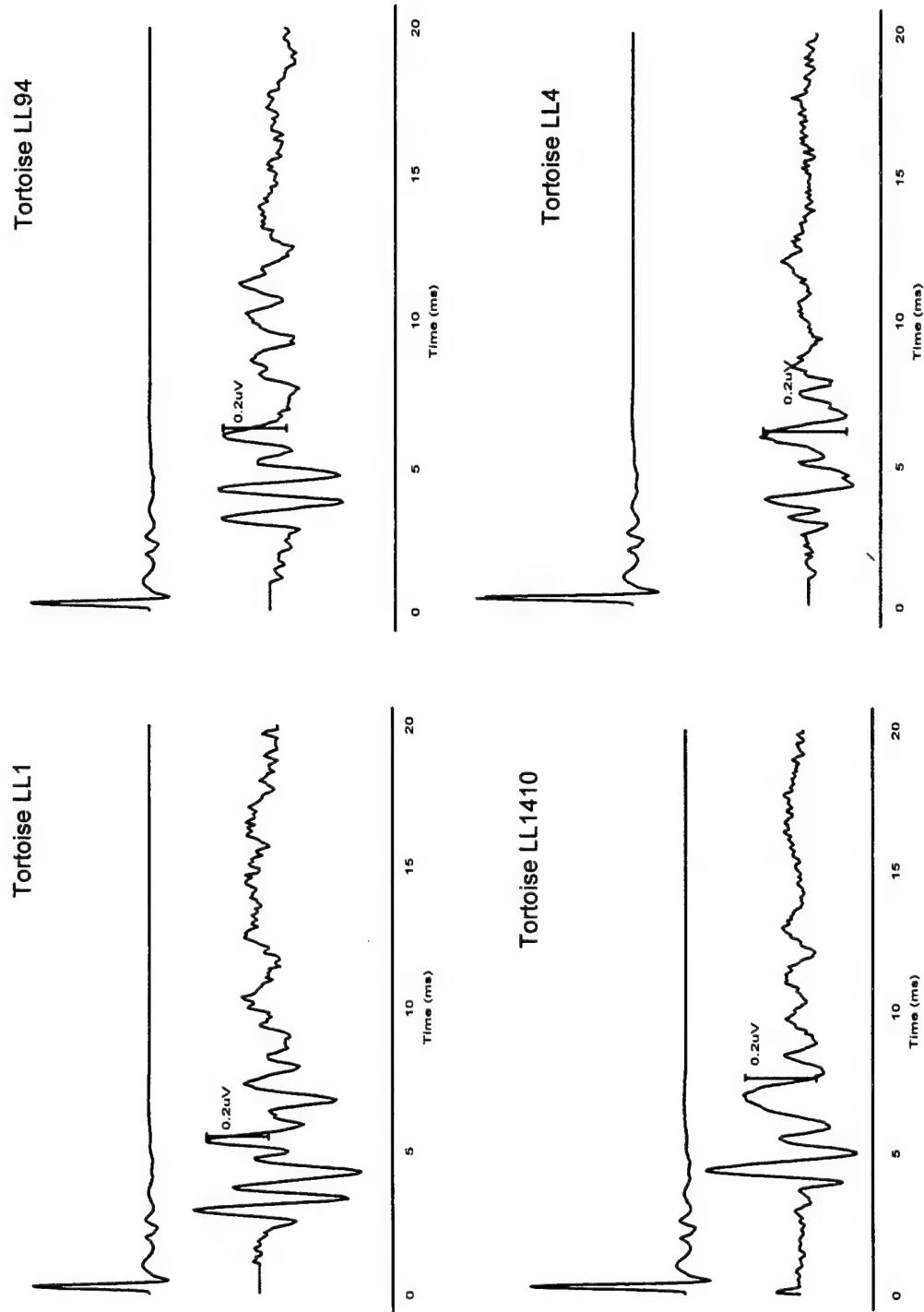


Figure 19. ABR waveform measurements elicited from four different tortoises by the click stimulus.

individuals. These occurred at latencies of 3-8 ms after stimulus onset. Three further peaks at latencies of 8-15 ms appeared in the ABRs of some individuals but not others (Figure 19). At high stimulus levels, ABR peak-peak amplitudes ranged around 0.5  $\mu$ V against a background consisting of electrical system and electrophysiological noise with a peak-peak amplitude of around 0.1  $\mu$ V (Figure 20).

Figure 21 shows the waveforms of a single tortoise recorded during 5 separate experiments using the 250 Hz tone pip. The tone pip stimulus at this frequency was 20 ms long and overlapped the returning AEP considerably. The waveform was closer in structure to a frequency-following response than an ABR (note the difference between waveforms in Figure 19 and 21). The waveform varied from experiment to experiment much more than the click-evoked ABR. This variability was the result of cross-talk between the input and output modules of the Biologic measurement system, which introduced an artifact that could not be removed. Therefore, the results of the tone pip measurements were more difficult to interpret than those collected using the clicks. However, this artifact disappeared at stimulus levels  $< \sim 50$  dB and did not prevent threshold measurements from being collected. It did, however, introduce noise that produced odd phase shifts in the AEP waveforms (e.g., Figure 21, Record 25).

Latency shifts with decreasing stimulus amplitude were small for the desert tortoise (Figure 22), on the order of 1 ms from the highest to the lowest signal levels (~80 down to ~20 dB). Peak latency was also very difficult to measure at low stimulus levels due to the relatively low signal to noise ratio of tortoise ABR/AEP waveforms. Latency shifts were not collected as a measure of changes in threshold.

Figure 23 shows the thresholds collected during this study in the range from 250-2000 Hz for all

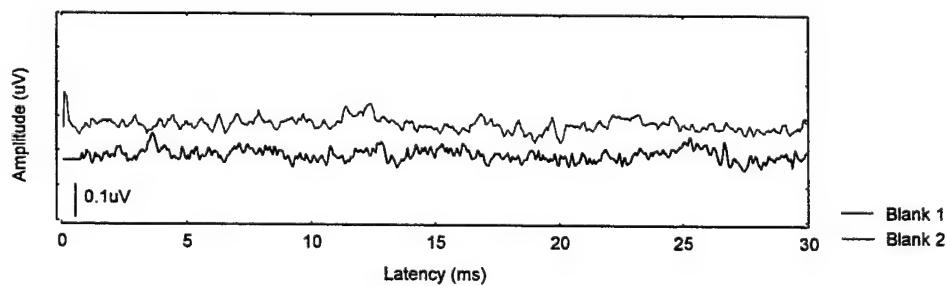


Figure 20. ABR waveforms of a desert tortoise in the absence of test stimuli (blank trials).

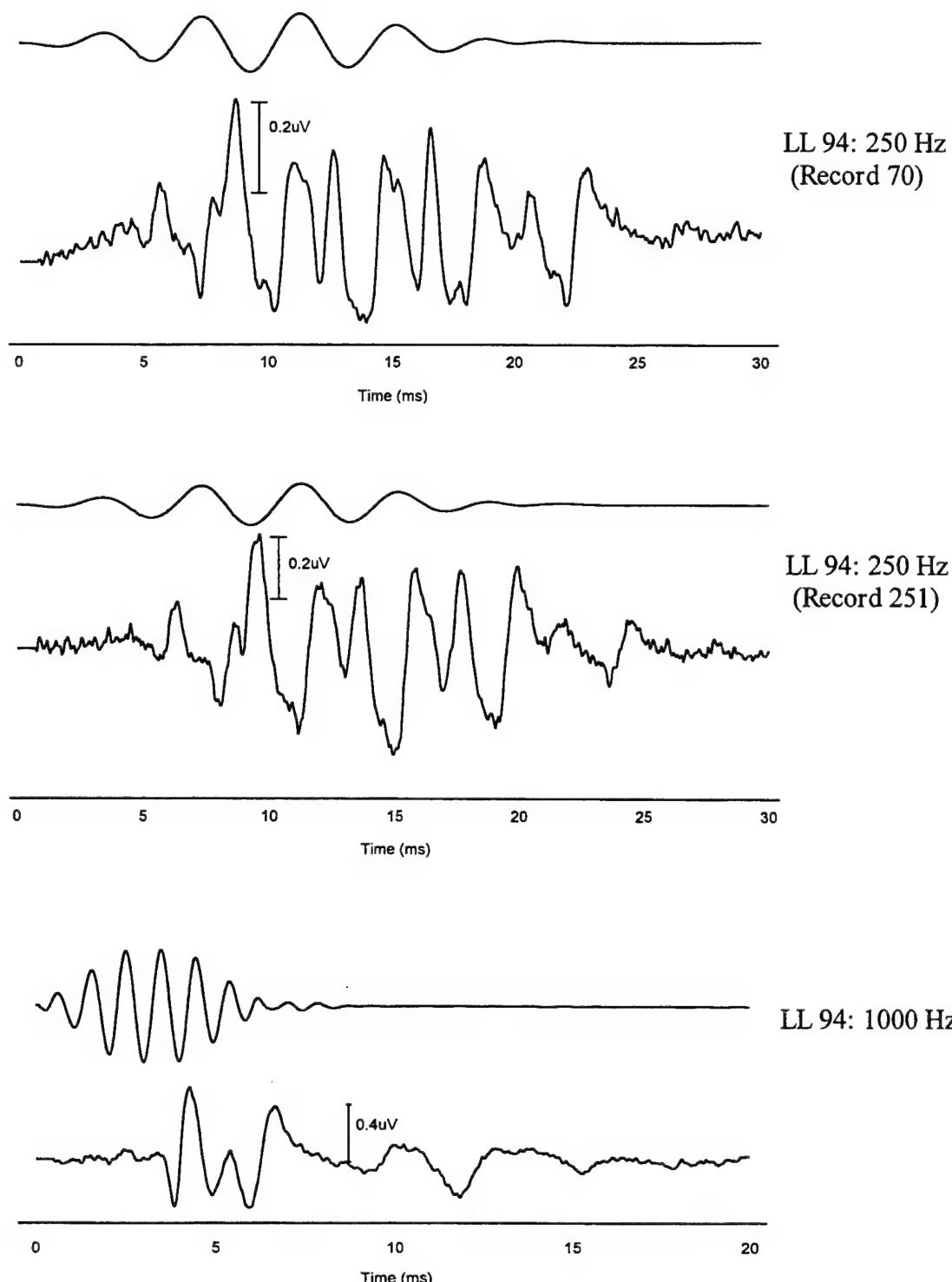


Figure 21. Top, Middle: AEP waveforms of desert tortoise 94 elicited by 250 Hz tone pips; the ABR waveform is detectable, but obscured by a system artifact. Bottom: at higher frequencies, this artifact no longer obscures the ABR.

Table XII. Thresholds of desert tortoises estimated using ABR/AEP measurements. Free-field measurements refer to experiments in which the stimuli were projected using a Bose woofer speaker. Insert-ear measurements were collected using the ER-2 insert ear speaker. If more than one value was collected for an individual tortoise, the average was given. The first column gives the average for all tortoises. Data on *Pseudemys scripta* were taken from Patterson (1966; as summarized in Fay 1988).

Frequency	Ave	LL1	LL4	LL6	LL15	LL32	LL42	LL47	LL52	LL54	LL93	LL94	LL1355	LL1410	Pseudemys
<i>Free-field</i>															
20															101.7
50	76.25		70.00								82.5				
64															66.3
80	55.00	57.50								72.5	42.50	47.50			
125	49.50	47.50		45.50					67.50		37.50				
200															42
250															
500															
<i>Insert ear</i>															
Click	50.62	42.50	46.67	57.50	46.25	47.50	62.50			52.50	61.25	46.79	47.50	45.83	
200															42
250	30.78	27.50	25.00	35.00	27.50	27.50	42.50			39.38	35.83	25.000	27.50	25.83	
400															41.3
500	42.47	32.50	35.00	47.50	43.75	42.50	65.00	42.50	52.50	50.00	53.33	22.50	37.50	27.50	
640															
800															
1000	64.83	58.73	61.67	70.00	64.17	57.50	62.50	67.50	72.50	60.00	70.83	59.50	73.750	64.17	54.3
2000	84.20		85.00	77.50	82.50	87.500	85.00	92.50	88.75	90.00	75.00		85.00		

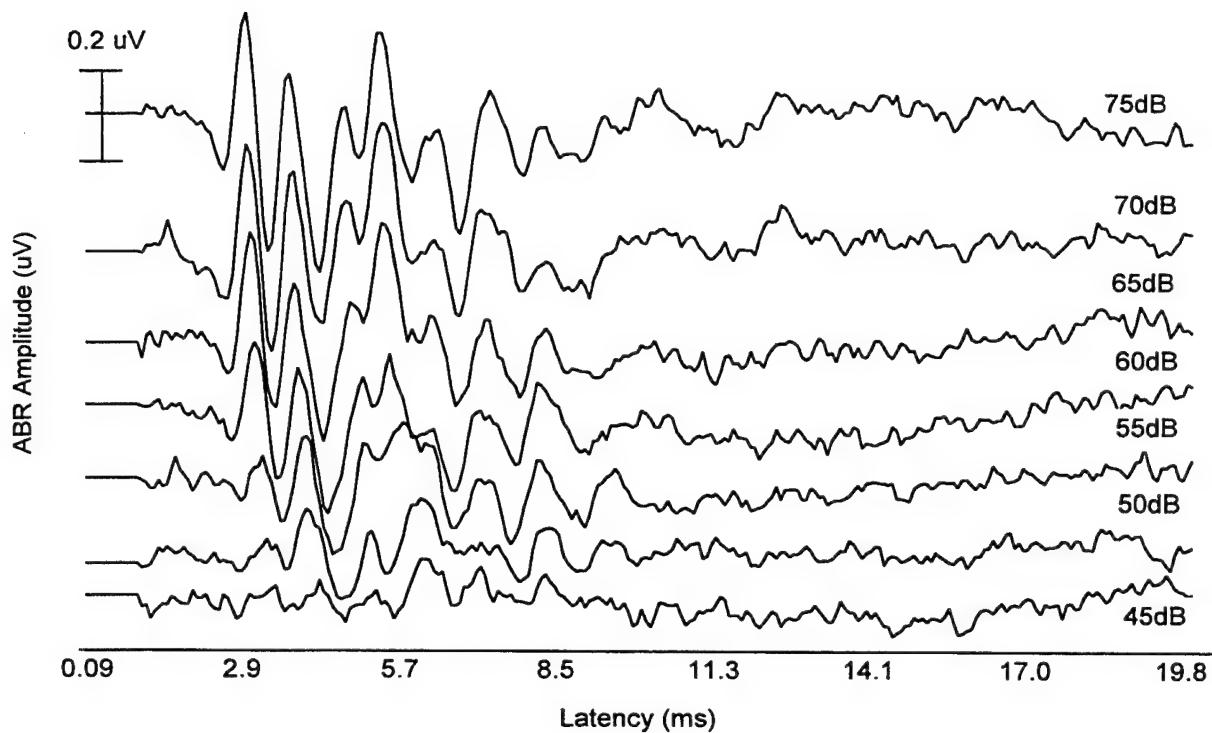


Figure 22. Amplitude and latency shifts in click-evoked ABR waveforms with decreasing stimulus level. Amplitude decreased with decreasing level, but latency shifts were small.

tortoises combined (see also Table XII). The range of thresholds detected at each frequency was 30-40 dB wide among tortoises. Within tortoises, variation in threshold for measurements collected within a few days of each other was 5-10 dB. However, larger differences were detected over the course of the study.

The source of the interindividual variability was two-fold. First, there was doubtless some difference in actual hearing sensitivity. However, there were also tempermental and behavioral differences among individuals. Some tortoises, for example LL94, were always relaxed when being tested, making measurements easier to collect. Within-individual variability was the result of small changes in temperature over the course of the experiments, animal movement, and small variations in experimental technique. A number of experiments were eliminated during analysis because electrical noise or animal movements made ABR peaks difficult to detect. Some individuals were particularly prone to yield such failures, specifically LL1522 and LL47.

Measurements of threshold at high frequencies ( $> 2000$  Hz) required stimulus levels high

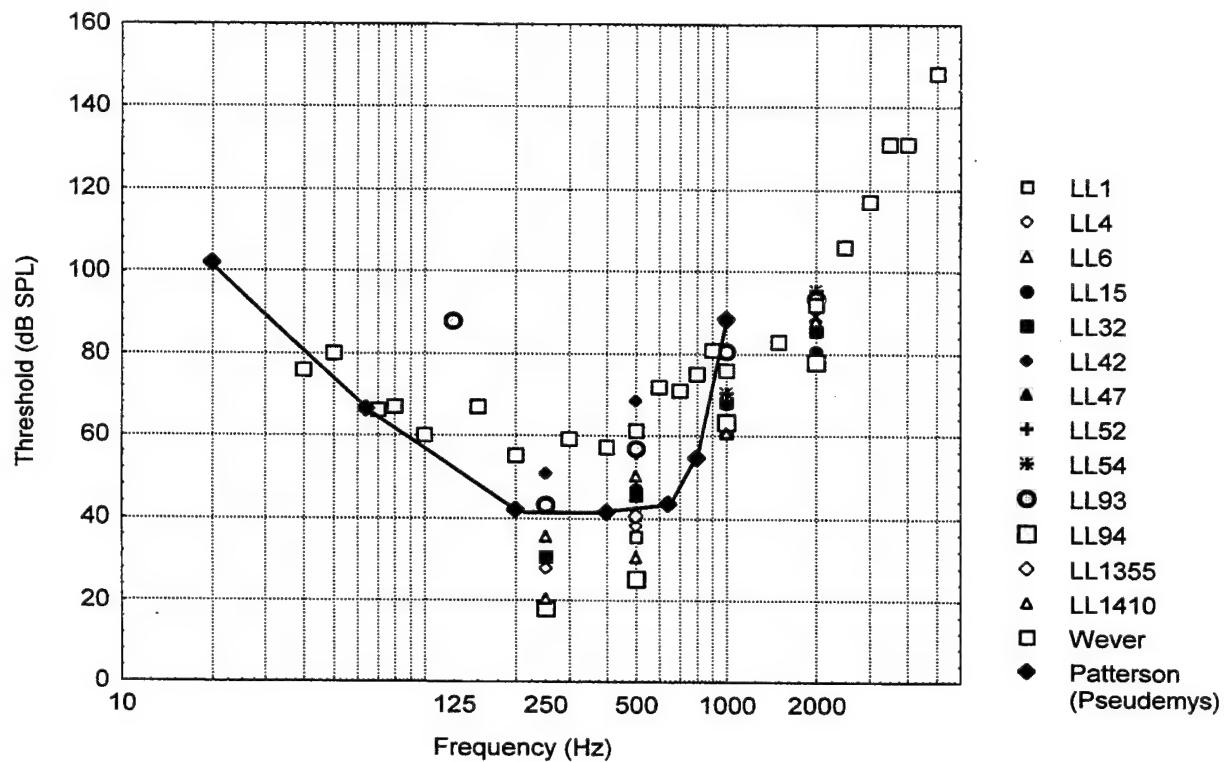


Figure 23. Thresholds estimated for test tortoises at frequencies from 250 to 2000 Hz, plotted against Patterson's 1966 behavioral auditory thresholds for the pond slider (*Pseudemys scripta*) and Wever's (1978) electrophysiological thresholds for the Eastern box turtle (*Terrapene carolina*).

enough that non-linearities in the Bio-logic system were a factor in the accuracy of measurements. These data were eliminated and 2000 Hz was taken to be the upper frequency limit for the species. These results were consistent with previous electrophysiological measurements collected from the cochlea, and indicated somewhat better sensitivities at high frequency than Patterson's behavioral curve for *Pseudemys scripta* (Figure 24).

The average sensitivity at best frequency (250 Hz) for the tortoises was over 10 dB better than expected (Figure 24); of the 11 tortoises for which best sensitivity was measured in this study, most had estimated thresholds lower than Patterson's average at best frequency. The most sensitive had an average threshold of 25 dB. During the playback experiments, when temperature conditions were optimal and when the tortoises were well-habituated to the experimental procedures, 4 of the tortoises had measured thresholds of 17.5 dB. Measurements collected during these repeated experiments were extremely repeatable (error of 0-5 dB). The threshold of

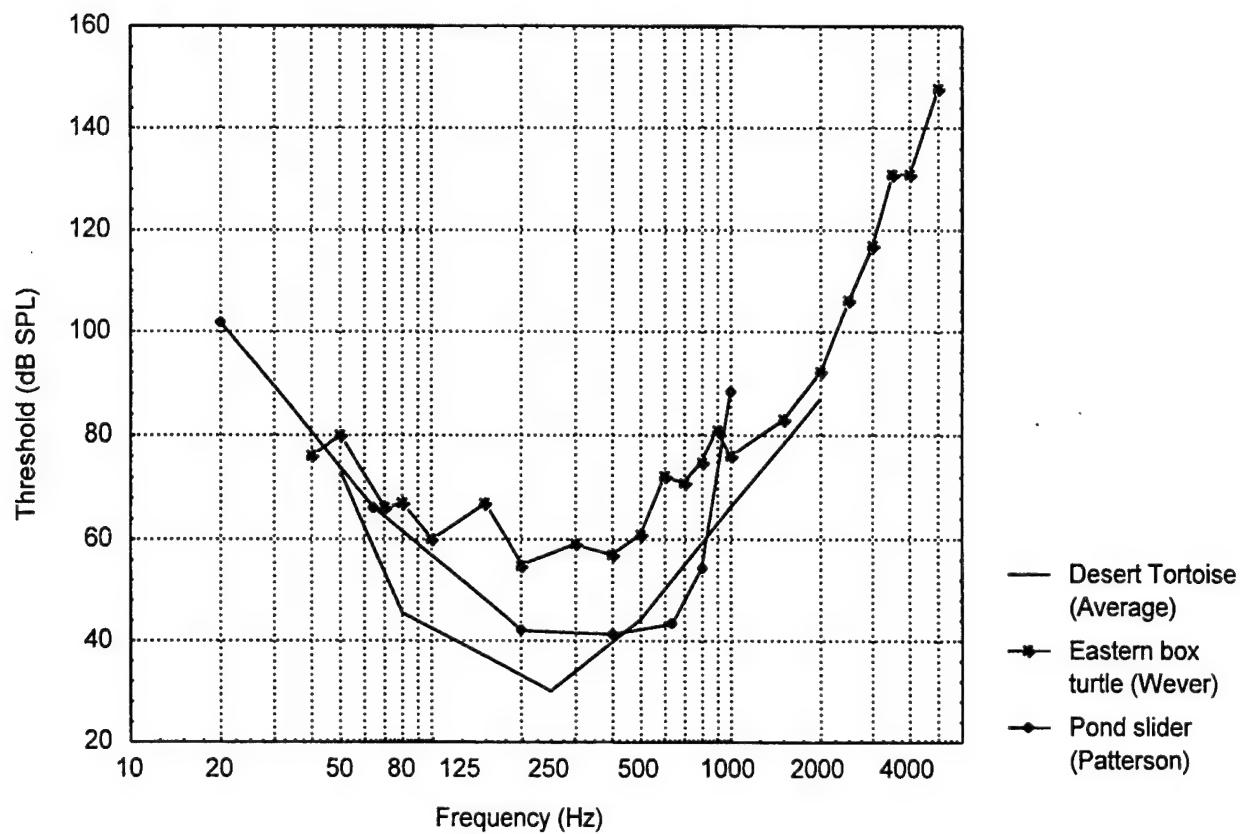


Figure 24. Average of thresholds for all test tortoises, plotted with Wever's (1978) electrophysiological thresholds for the Eastern box turtle and Patterson's (1966) behavioral auditory thresholds for the pond slider. Free-field, low-frequency auditory thresholds included.

17.5 dB was close to the estimated noise floor of the measurement system.

There was a modest but significant correlation between carapace length and sensitivity (Pearson product-moment correlation,  $r = 0.33$ ,  $p < 0.05$ ), suggesting that tortoise hearing varies with age.

### Effects of simulated aircraft noise on hearing

Six desert tortoises were exposed to the 40 minute jet playback tape (Table XIII). Four were exposed to two 6-psf booms in the INTF (Table XIV). ABR thresholds were measured in response to clicks and to tone bursts at 250 and 1000 Hz before and after each exposure. Thresholds after exposure differed from the before exposure condition. However, they *decreased* by an average of 3.2 to 5.7 dB - that is, the tortoises were apparently more sensitive after exposure vs. before.

The reasons for this difference were not completely clear. Conditions before and after the exposures differed in two important ways. First, tortoises were exposed to marginally higher temperatures in the sound isolation chamber during the post-playback condition (difference  $\sim 2^{\circ}\text{C}$  ). To minimize differences due to temperature shifts, ABR measurements were made using click stimuli immediately before and after exposures. Nevertheless, temperature differences could have been a factor. Second, as will be clear from the description of behavioral playback experiments below, exposure to high sound levels suppressed activity of the tortoises in many cases; this made tortoises less likely to awaken and move during the post-exposure measurements. Both conditions tended to result in slightly lower threshold estimates.

Figures 25 and 26 show the threshold measurements collected during the baseline period (stars; collected in the 6 mo prior to the start of playback experiments), those collected just before exposure (open boxes), and those collected immediately after (solid diamonds). The range of these values overlapped completely and the sample sizes were small; therefore statistical comparison yielded no detectable differences.

Table XV summarizes threshold shifts during experiments with 10 6-psf simulated sonic booms. Unlike the case for subsonic aircraft noise and trials with two 6-psf booms, most of the shifts were positive (that is, the tortoise was less sensitive after exposure). Average shifts were small but positive (1.5-6.1 dB) and seemed to increase from the first to the second measurement using clicks, suggesting that threshold shift was greatest at an hour or more after exposure. Except in the case of the largest shift (LL54), these changes were difficult to detect during the experiments. Therefore, only LL54 was deliberately retested soon after exposure. Tortoises could not be safely sedated more often than once in 48 hours; therefore, retesting occurred at  $\sim$ 48 hours after the exposure. Evidence of recovery was found in LL54, but recovery may not have been complete (thresholds of 5-10 dB above the pre-exposure measurement, down from a shift of 20 dB).

Table XIII. Comparison of averaged ABR thresholds during experiments with subsonic aircraft noise. Table lists the best sensitivity measured for each individual and the difference between pre and post-exposure thresholds at each stimulus frequency (clicks, 250 Hz tones, and 1000 Hz tones).

Tortoise	Date	Best Sensitivity* (dB)	Test Stimulus			
			First Click	250 Hz	1000 Hz	Second Click
LL1	9/15/95	27.5	0.0	-5.0	0.0	0.0
LL4	9/14/95	22.5	0.0	0.0	-5.0	0.0
LL32	9/18/95	37.5	-4.5	-5.0	-5.0	-
LL54	9/8/95	27.5	-15.2	0.0	0.0	-
LL94	9/11/95	17.5	-5.0	0.0	0.0	-
LL1410	9/15/95	42.5	-9.5	-20.5	-15.5	-9.5
Average Shift			-5.7	-5.1	-4.2	-3.2

\* level in dB of least detectable ABR at 250 Hz in the pre-exposure measurement.

Table XIV. Comparison of averaged ABR thresholds during experiments exposing tortoises to 2 6-psf simulated booms. Table lists the best sensitivity measured for each individual and the difference between pre and post-exposure thresholds at each stimulus frequency (clicks, 250 Hz tones, and 1000 Hz tones).

Tortoise	Date	Best Sensitivity (dB)	Test Stimulus			
			First Click	250 Hz	1000 Hz	Second Click
LL6 <sup>1</sup>	9/19/95	32.5	-	-	-0.5	-
LL42	9/20/95	37.5	-5.0	-5.0	-5.0	-
LL54	9/22/95	32.5	-15.0	-15.0	-7.0	-
LL94	9/20/95	17.5	0.0	-0.5	-5.0	-
Average Shift			-6.7	-6.8	-4.4	-

\* level in dB of least detectable ABR at 250 Hz during the pre-exposure measurement.

<sup>1</sup> No good-quality ABR data were obtained from this tortoise. Differences were obtained by subtracting from data collected at an earlier date.

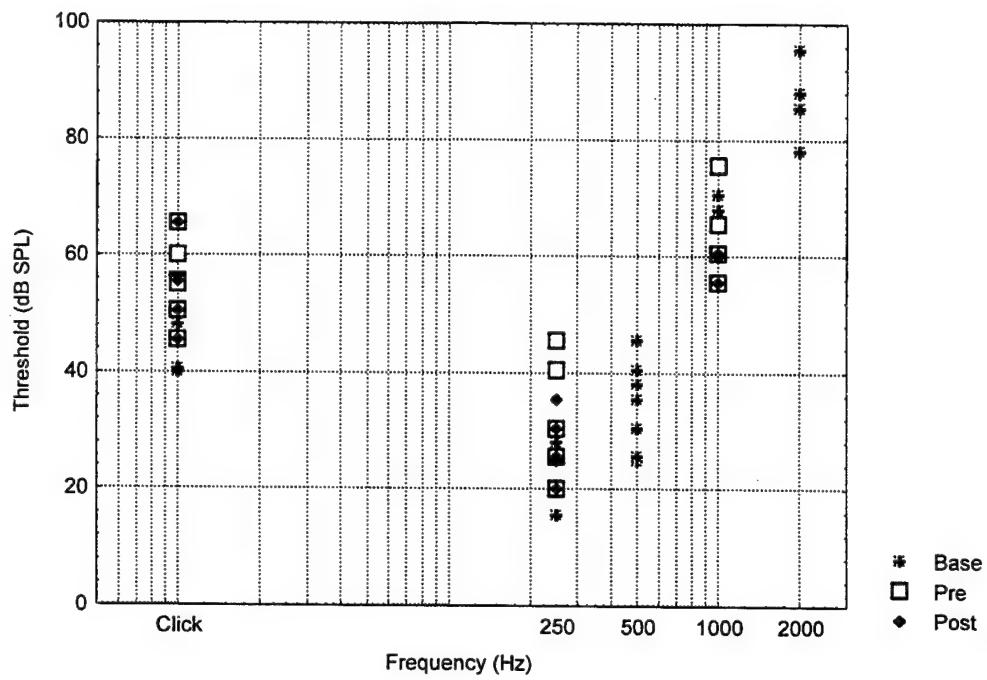


Figure 25. Comparison of averaged ABR thresholds during experiments with subsonic aircraft noise. Figure shows thresholds during baseline, pre-exposure, and periods.

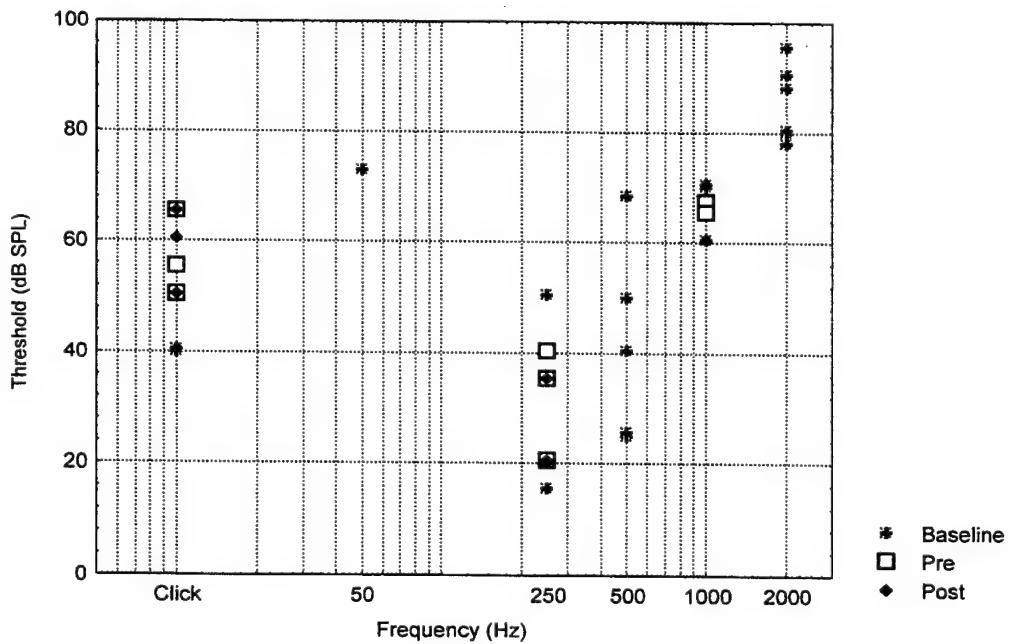


Figure 26. Comparison of averaged ABR thresholds during experiments with 2.6-psf sonic booms. Figure shows thresholds during baseline, pre-exposure, and periods.

Table XV. Comparison of averaged ABR thresholds during experiments exposing tortoises to 10 6-psf simulated booms. Table lists the best sensitivity measured for each individual and the difference between pre and post-exposure thresholds at each stimulus frequency (clicks, 250 Hz tones, and 1000 Hz tones).

Tortoise	Date	Best Sensitivity* (dB)	Test Stimulus			
			First Click	250 Hz	1000 Hz	Second Click
<b>LL4</b>	11/1/95	27.5	0.0	5.0	5.0	5.0
<b>LL6</b>	10/26/95	37.5	-5.0	-10.0	-10.0	5.0
<b>LL15</b>	10/30/95	32.5	0.0	-5.0	0.0	0.0
<b>LL32</b>	10/27/95	17.5	10.0	0.0	5.0	5.0
<b>LL54</b>	11/1/95	32.5	20.0	20.0	20.0	>20.0
	Retest, 11/4/95	32.5	5.0	0.0	10.0	5.0
<b>LL93</b>	10/29/95	17.5	0.0	-10.0	0.0	-5.0
	11/9/95	17.5	5.0	0.0	-	5.0
<b>LL94</b>	10/25/95	17.5	0.5	-0.5	-5.0	-
<b>LL1355</b>	10/30/95	27.5	5.0	10.0	-10.0	5.0
<b>LL1410</b>	10/25/95	17.5	5.0	0.0	0.0	-

\* level in dB of least detectable ABR at 250 Hz immediately before exposure.

The remaining shift (5-10 dB) was within measurement error and was probably not significant, as within individual-variability from measurement to measurement is similar in other species (humans and laboratory animals). As an example, differences between pre- exposure and baseline experiments averaged 8 dB at 250 Hz and 9 dB at 1000 Hz, probably the result of differences in experimental conditions (temperature, tortoise movement).

In the case of LL54, the change in threshold (20 dB decrease in sensitivity) was well outside experimental error; therefore, the shift was significant for this individual (Figure 27). The change was very obvious on an examination of the tortoise's ABR. Before experiments, it was easily recognized. In the hour after exposure, the peaks were reduced to the point where they were difficult to measure. Two days later, the waveform was no longer distinguishable from the pre-exposure condition (Figure 28). The changes in the waveform immediately after exposure eliminates any possibility that the shift could have been the result of error in the measurement of the least detectable ABR.

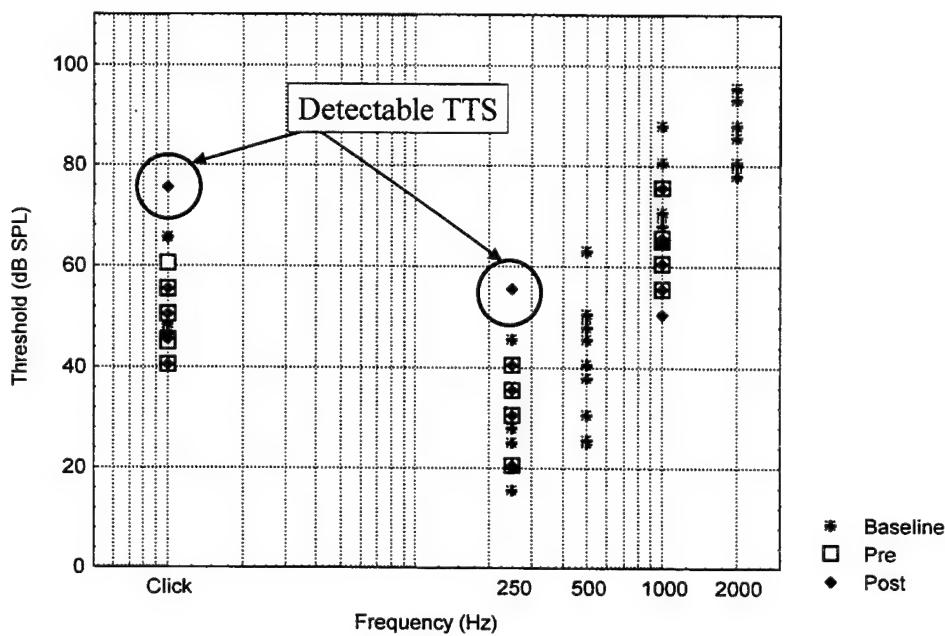


Figure 27. Comparison of averaged ABR thresholds during experiments exposing tortoises to 10 6-psf booms. Figure shows thresholds during baseline, pre-exposure, and periods.

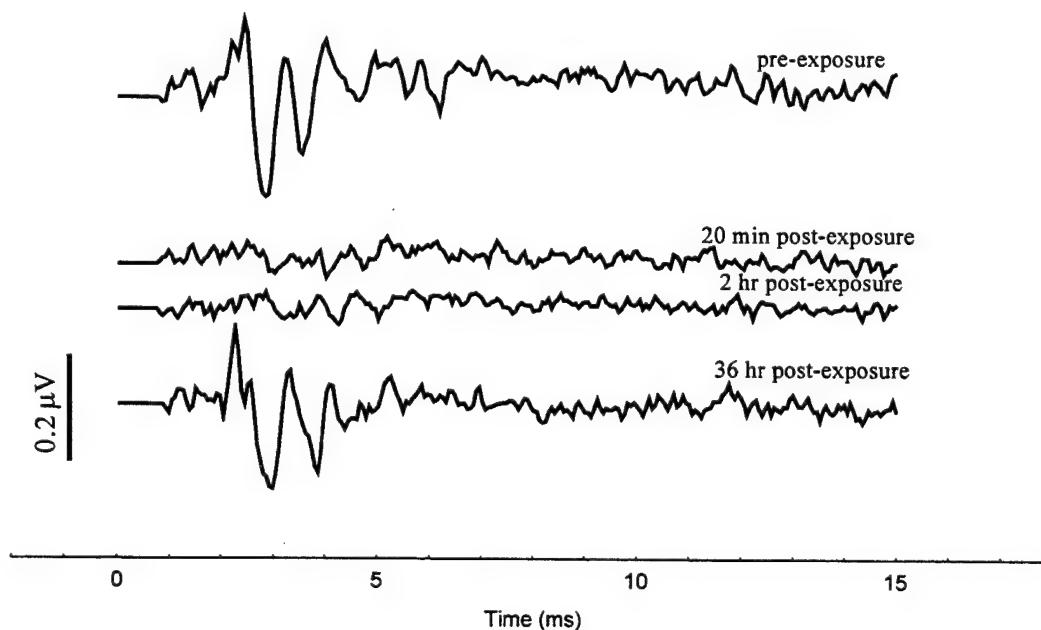


Figure 28. Click-evoked ABR at 70 dB SPL for tortoise LL93 before, 20 min after, 2 hr after, and 36 hr after exposure to 10 6-psf booms.

Based on these results, only one tortoise showed a significant threshold shift as a result of exposure to 10 6-psf booms. Note however, that smaller, undetected shifts may have occurred in other tortoises. Figure 29 shows the distribution of positive and negative shifts. Changes observed were consistently positive in 7 of the 9 individuals tested with the 10-boom protocol, as opposed to being consistently negative in experiments with subsonic aircraft noise and the 2-boom protocol.

Latency shifts might also have been a sensitive indicator of threshold shift even in cases where amplitude shifts were not evident. Unfortunately, little evidence of change in latency with signal amplitude was detected in the desert tortoise. ABR waveforms collected before and after exposures to noise in the most sensitive tortoise, LL54, showed no evidence of additional latency shift.

#### Relationship between sensitivity and temperature

An examination of temperature effects on sensitivity was outside the scope of this program.

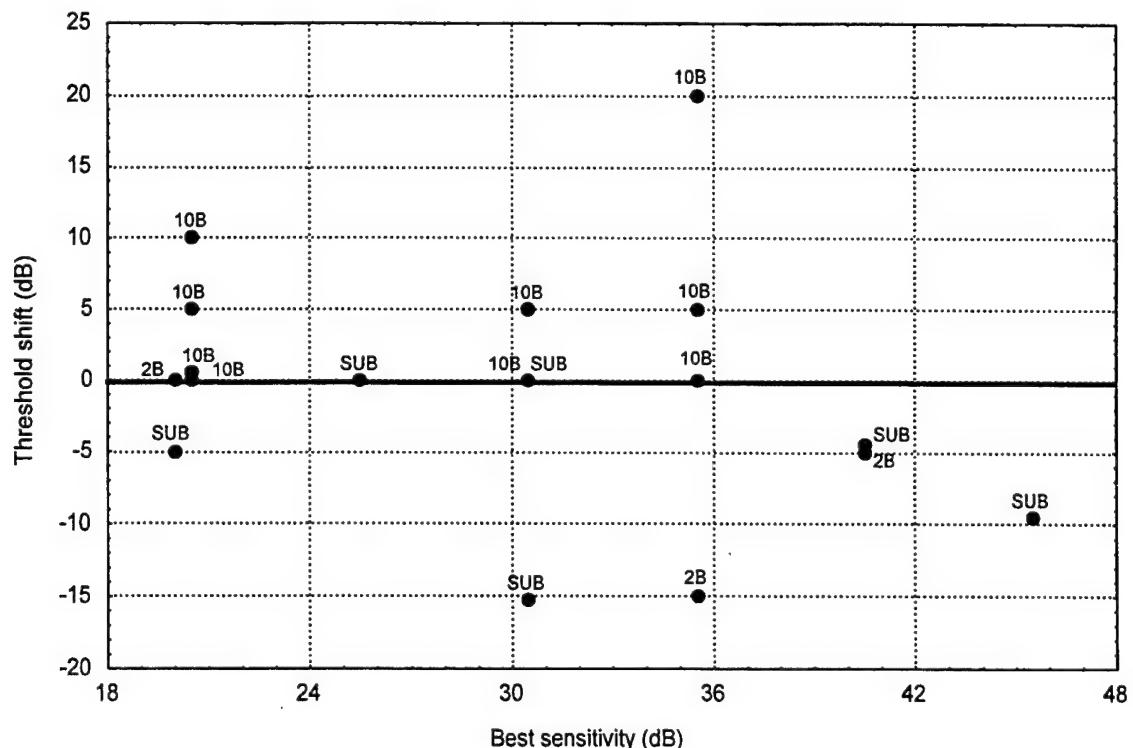


Figure 29. Threshold shifts of desert tortoises exposed to simulated subsonic noise (SUB), two 6-psf booms (2B), and 10 6-psf booms (10B). In the absence of significant threshold shifts, values should have been centered on 0 dB of shift.

However, investigators were concerned that the results of this study could have been compromised if tortoises were not at their most sensitive at test temperatures. Therefore, the hearing of one tortoise (LL54) was examined over a range of temperatures (24.7, 27.1, 28, and 32°C). As reported by Wever (1978) for other testudinates, the relationship between temperature and sensitivity was not expected to be linear, but rather a threshold function. Above a critical temperature, hearing sensitivity would be maximal; below it, the tortoise was expected to become increasingly insensitive.

High amplitude ABR waveforms (60-80 dB) were examined to determine amplitude and latency of each ABR peak. Based on the small amount of data collected, at best frequency (250 Hz), there was an obvious correlation between temperature and ABR amplitude (Figure 30). Below a critical temperature range (27-28°C), ABR amplitude was low. Above it, there was a marked increase in ABR amplitude. For ABR peaks P2, P3 and P4, usually the most obvious peaks in the tone-pip evoked waveform, the increase was more than one order of magnitude. The change in amplitude with temperature suggested that all data were collected in the temperature range within which tortoises are most sensitive.

There was also evidence that latencies of peaks in the waveform decreased with increasing temperature, as might be expected if the tortoise had greater sensitivity at high temperatures (Figure 31).

These changes in amplitude and latency were difficult to correlate to best sensitivity, as the best sensitivity of this tortoise was often difficult to ascertain.

#### Sensitivity to low-frequency tone pips

As discussed in the introduction, low-frequency ABR measurements are difficult to make, even in the absence of electrical artifacts. During these experiments, ABR measurements using the insert ear speakers (ER-2 and ER-3) proved to be very unreliable at frequencies below 250 Hz. One possible explanation for the poor responses was inadequacy of the stimulus itself - at low frequencies, the signal from a tiny 2 mm speaker is a very poor simulation of a free-field stimulus. Therefore, a series of ABR experiments were conducted using a Bose woofer speaker to deliver the stimulus to the tortoises in a free field. In this case, thresholds represented the sensitivity of the tortoise to a signal delivered at both ears.

The noise floor in the IAC chamber was higher than the noise floor within the ER speaker-rubber cover assembly; therefore, thresholds at higher frequencies (250, 500 Hz) might not have been as accurate. However, thresholds collected in the free field at these frequencies proved

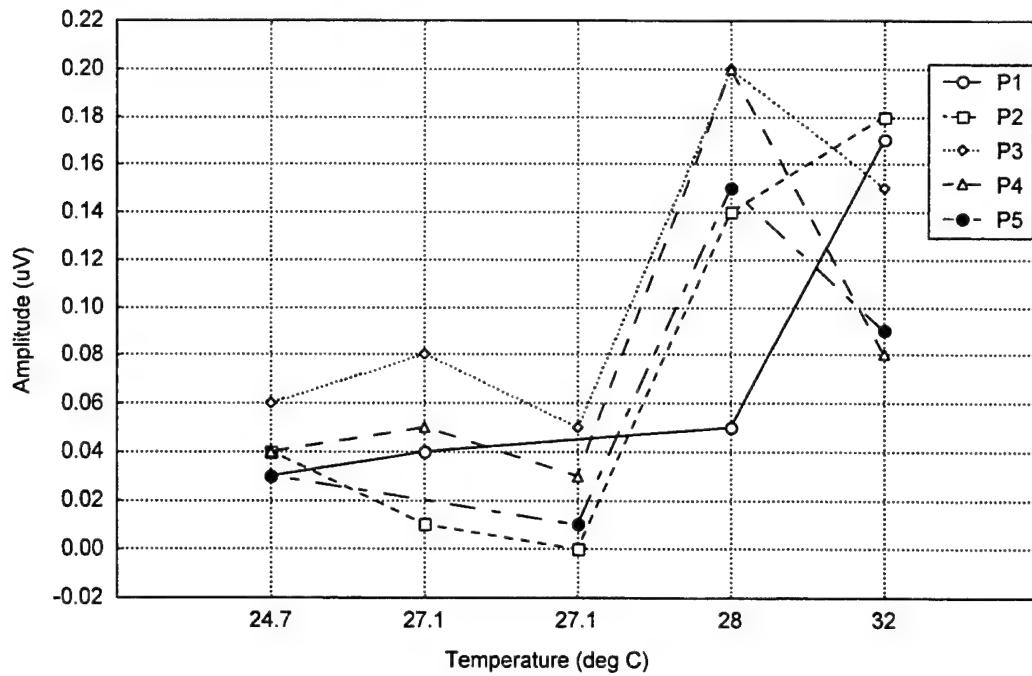


Figure 30. Relationship between amplitude of ABR peaks and temperature. The ABR waveforms were elicited by high amplitude (60-80 dB SPL) tone pips at 250 Hz. Note: two separate measurements were conducted at 27.1 °C.

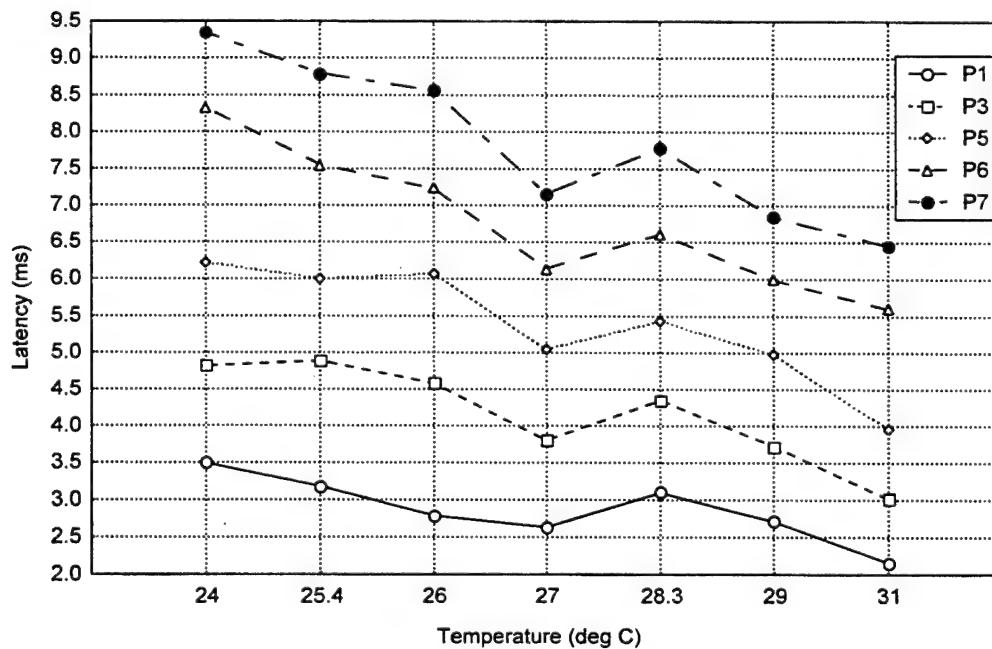


Figure 31. Relationship between latency of ABR peaks and temperature. The ABR waveforms were elicited by high amplitude (60-80 dB SPL) tone pips at 250 Hz.

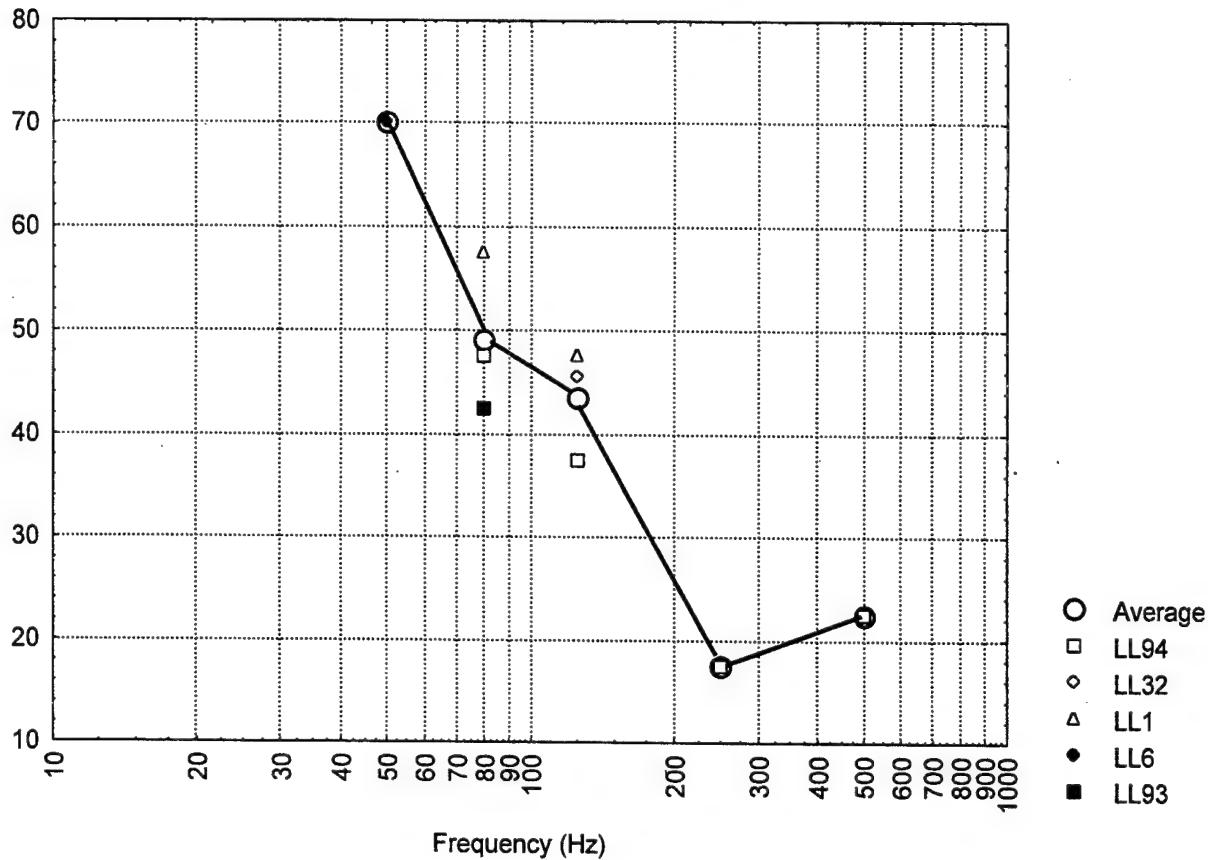


Figure 32. Low-frequency auditory thresholds of indicated tortoises collected by presenting tone pips in a free-field.

to be similar to those collected using the ER-2 speaker during the same period (Figure 32). At lower frequencies, the tortoises were less sensitive, and signal to noise ratios of ~10 dB could be achieved. Therefore, these low frequency thresholds were unlikely to be noise limited (Figure 32).

Tortoise sensitivity rolled off very rapidly below 250 Hz (~17 dB/octave).

#### Envelope-following responses

Tortoise FFR waveforms were elicited using continuous, amplitude-modulated (AM) tones (Figure 14). FFRwaveforms matched the waveform of the eliciting AM signals reasonably well in three successful experiments out of 7; in the rest, crosstalk between the input and output modules of the Bio-logic system contaminated the signal. FFT spectra were calculated from the

FFR waveforms when the match to the input signal appeared good. These waveforms were probably contaminated by crosstalk as well, because the spectra showed peaks not only at the carrier and modulation frequencies, but at many sideband frequencies as well. Nevertheless, a spectral peak could be detected at the modulation frequency and this peak changed in amplitude with input stimulus level. During analysis, the amplitude of this peak was measured and plotted against stimulus levels from 90 down to 45 dB SPL (the noise floor in the system).

Figure 33a shows the relationship between stimulus level for a 750 Hz tone modulated at 125 Hz graphed against the amplitude of the FFT peak at 125 Hz. To produce the figure, peak levels were measured repeatedly (10 times) and the range (minimum to maximum) was graphed. At high stimulus levels, the measurements had a small range; below about 75 dB, the range increased greatly. This was the cleanest of the three functions obtained. The second best is shown in Figure 33b for an 800 Hz tone modulated at 80 Hz. In both cases, an estimate of threshold could be obtained (75 dB in Figure 33a and 80 dB in Figure 33b) however, neither estimate agreed well with the results of free-field measurements (Figure 32) on the test tortoises, or with Patterson's (1966) data for the pond slider.

#### Otoacoustic emissions

Otoacoustic emissions could not be measured successfully from the desert tortoises. Audiogram measurements showed only minimal signals (at best 13 dB above the background and never consistently measurable) across the GM frequency range from 250-2000 Hz (i.e., the range within which the tortoises were expected to hear well), even with primary stimulus levels high (70-80 dB SPL). SNR measurements were always low, but seemed to be best for a frequency ratio of  $r=1.41$ . Suppression curves could not be measured successfully, suggesting that the sounds measured were not OAE returns. At 250 Hz, the estimated threshold for the only animal that yielded useful results (LL42) was 50 dB; this animal's best threshold as measured by ABR was 32.5 dB. In addition, the threshold as measured in selected frequency bins ( $2f_1-f_2$ ,  $3f_1-2f_2$ ,  $2f_2-f_1$ ,  $f_1-f_2$ ) was never consistent, as it would have been if a strong response had been obtained. Efforts to make measurements using this technique were abandoned after a few experiments.

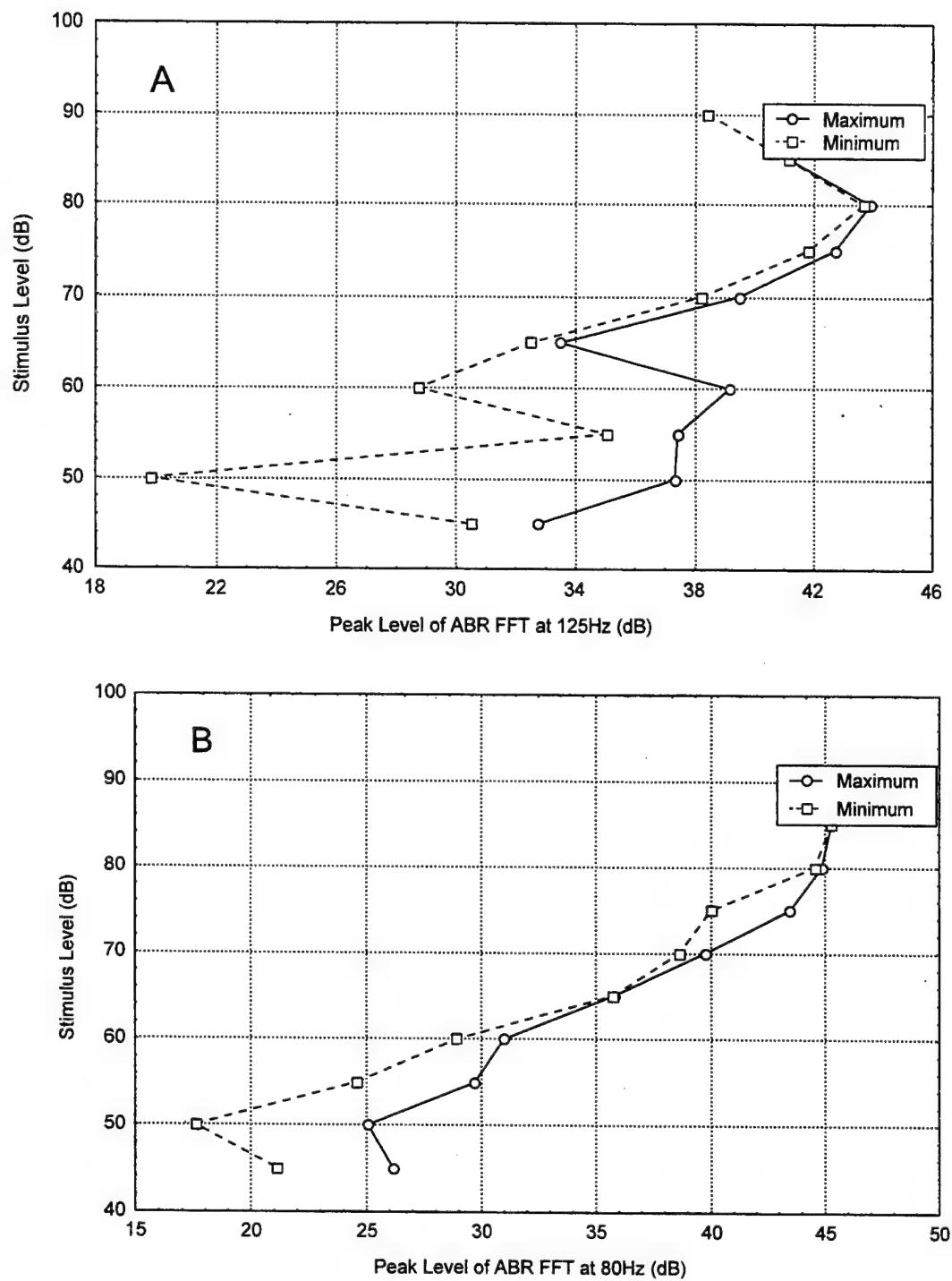


Figure 33. Results of envelope following response (EFR) measurements. (A) Level of spectral peak at 125 Hz plotted against stimulus level (carrier frequency: 750 Hz; modulation frequency: 125 Hz). Peaks were measured repeatedly; the minimum and maximum reading were plotted. (B) Level of spectral peak at 80 Hz (carrier frequency: 800 Hz; modulation frequency: 80 Hz).

## Behavioral Responses

### Responses to simulated low-altitude overflights

Desert tortoises displayed a range of responses to initial exposure to aircraft overflights. The most obvious and intense of these was freezing. The longest freezing bout lasted 113 min, well after the end of the 44 minute exposure period, but this was atypical. Typically, freezing took the form of an abrupt cessation of activity, after which the tortoise either remained still, or looked around periodically while remaining in place. Within ~10 min of exposure, it began to move about again.

Based on intuition, it might be expected that tortoise freezing and other responses would follow immediately upon the onset of exposure. However, such close connection in time was not usual. This raised the possibility that tortoise 'reactions' were actually normal behaviors that would have occurred anyway, regardless of the presence of the aircraft overflights. Therefore, detailed behavioral analysis was conducted of behaviors in the hour before, during, and after exposure to subsonic overflights. Since the duration of these behaviors was a crucial variable, care was taken to include the full duration of the initial and ending behavior in the analysis - that is, if the tortoise was still at the start or end of the observation, the duration assigned to the 'still' behavior included the period since the last change in behavior and until the following change, even if it meant starting the observation early or ending it late.

To render the process of analyzing video data practicable, individual behaviors were often categorized into states for analysis. For example, tortoises that climbed the barricade in an effort to escape often rested or looked around for brief periods in between bouts of climbing; during the analysis of most of the data, these behaviors (climbing, resting, looking) were lumped together under the category 'climbing'. However, one day of exposures (9/25/95, tethered tortoise, LL1) was analyzed by recording every event to insure that important details of responses were not being missed. In the 2.32 hr before, 2.59 hr during, and 3.01 hr after exposure, a total of 213 individual behaviors were observed.

The common behaviors were holding still while looking around (70 instances), holding still ('freezing', 10 instances), turning abruptly to look (21 instances), attempting to climb the barricade (61 instances), and persistently walking along the barricade, nosing and pushing it (18 instances; Table XVI). These behaviors occurred against a background of diurnal variability - there were distinct active periods (typically 0900-1100 hr and 1300-1500 hrs) during the day, followed by periods of quiescence. These periods were probably entrained somewhat by habitu-

Table XVI. Incidences of the most common behaviors observed during subsonic aircraft noise exposures of tortoise LL1 on 9/25/95.

Behavior	Before Exposure	During Exposure	After Exposure	Total
Still	1	6	3	10
Looking while still	28	24	18	70
Head retraction	1	3	2	6
Looking while moving	5	14	8	27
Climbs the barricade	25	19	17	61
Investigates the barricade	4	12	2	18

ation to husbandry activities, which usually took place during those periods. However, during the experiment, the test tortoise was disturbed by a human only once and then briefly (< 2 min) at 1030, when it was fed.

For the purposes of illustration, Figure 34 shows the incidence and duration of one behavior, looking. Note that the periods of exposure do not appear to elicit large, permanent changes in looking behavior. In the morning and at noon, looking appeared to last longer after exposure, whereas in the evening bouts of looking were shorter. Other behaviors seemed to be similarly uncorrelated with the exposures. Only bouts of patrolling the barricade appeared to increase dramatically in number during the exposure period for this tortoise.

To simplify the data reduction process, most behaviors were pooled into categories for further analysis. The following discussion is based on 262 bouts of behaviors collected from 23.25 hr of observations before, 20.25 hr during, and 18.45 hr after exposure to subsonic aircraft noise. Head retraction was one of the few behaviors that was identified as an individual event because it was an easily-recognized defensive response. It was expected to occur at short latency after exposure. However, during aircraft noise exposures, the behavior proved to be uncommon. It was particularly unlikely to occur during the first overflight exposure. It did, however, increase in incidence during successive overflights. A total of 26 retractions were observed, 18 of which occurred during exposures and 7 in the half hour afterwards. Unexpectedly, it did not occur with short latency (time from overflight onset to head retraction was > 60 s in most cases).

Overall, the tortoises exhibited more bouts of behaviors during exposure (4.84 bouts/hr vs. 2.97 in the hour before exposure). This increase was largely the result of nearly double the number

of bouts of holding still during the exposure (21 bouts before, 51 bouts during, and 27 bouts after; Table XVII) and the increase in head retractions. They also looked around somewhat more often. Bouts of walking, climbing and digging declined. Investigation of the barricades varied greatly among individuals and was eventually pooled with other walking bouts.

Consistent with the increase in incidence, the duration of bouts of holding still was significantly shorter during vs. before and after exposures ( $13.9 \pm 22.1$  min vs.  $29.9 \pm 26.4$  and  $28.6 \pm 31.4$  min; ANOVA,  $df=2, 96$ ;  $F=4.33$ ;  $p < 0.0157$ ). This was the only significant change in duration of bouts (bouts of looking, walking and climbing were also tested; ANOVA;  $p > 0.05$ ). Change in the duration of walking bouts was large ( $24.05 \pm 37.4$  during vs.  $8.2 \pm 10.2$  before and  $16.1 \pm 25.9$  after), but the difference was not significant ( $p > 0.05$ ). A difference of this magnitude was probably the result of an actual change in behavior, obscured in the statistical examination by a large correlation between the means and variances.

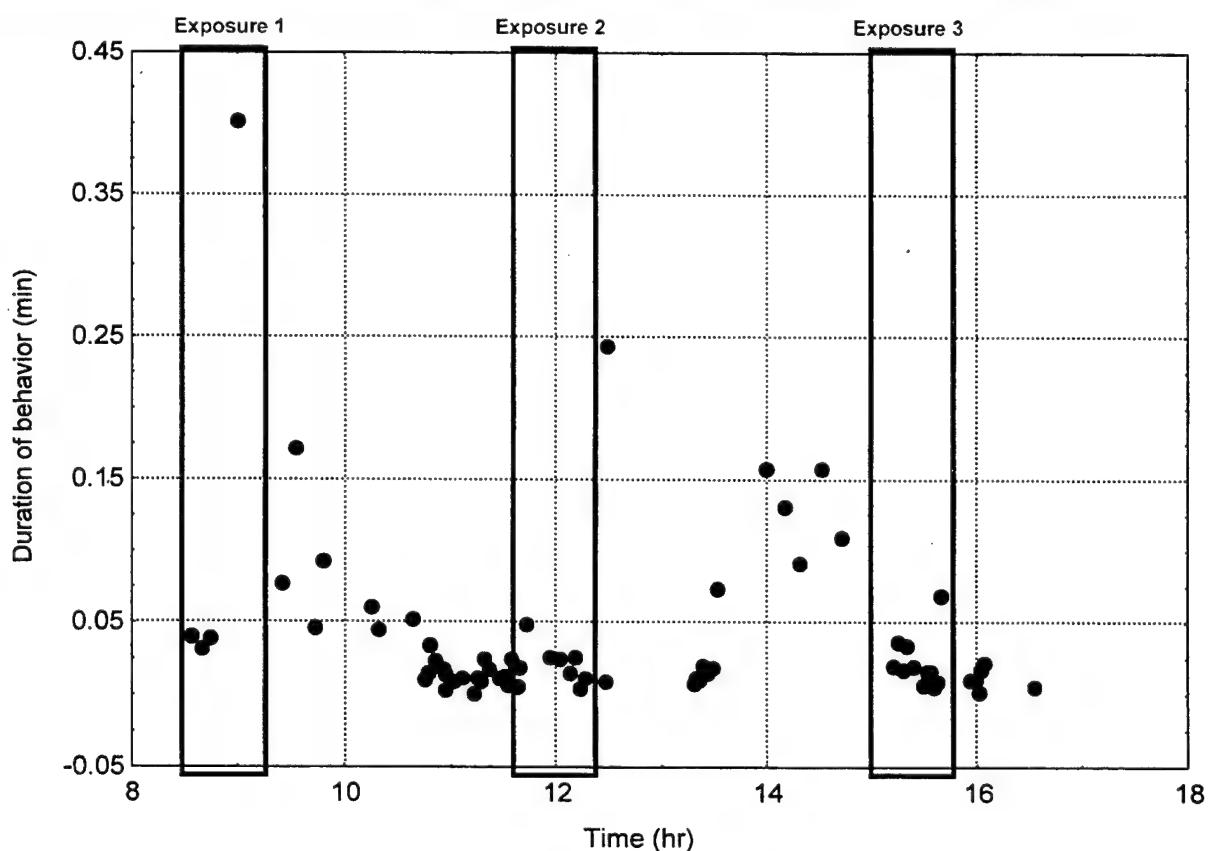


Figure 34. Example sequence of looking behavior (looking while still) exhibited by tortoise LL1 on 9/25/95 during exposures to subsonic aircraft noise.

Table XVII. Incidences of the most common behaviors observed during subsonic aircraft noise exposures (all tortoises). Looking, walking, climbing, and digging were scored as states (consisting of sequences or bouts of related behavioral events). Total observation time was the number of hours of observation scored to identify these behaviors. Rate was the number of behaviors per unit time.

Period	Before	During	After	Total
<b>Behavior</b>				
Still	21	51	28	100
Head retracted	0	15	5	20
Look	7	10	3	20
Walk	22	14	10	46
Climb	14	4	9	27
Digging	5	4	1	10
<b>Totals</b>	<b>69</b>	<b>98</b>	<b>56</b>	<b>223</b>
<b>Total Observation Time</b>	23.25	20.25	18.45	61.95
<b>Rate of Behavior</b>	2.97	4.84	3.03	

The quantitative differences were consistent with the impressions of observers, who saw the tortoises freezing (holding still) more often during exposures. To determine whether freezing was a consistent response to exposure, behaviors immediately before and after individual overflights were examined in sequences (Table XVIII a-e). For this analysis, the behavior immediately before and after each individual stimulus was quantified. The first exposure of a series, exposures during the series, and the last exposure of the series were treated separately. Viewed this way, bouts of holding still tripled in incidence before individual overflights during the overflight series *vs.* the periods before and after the overflight series or the period immediately after each individual overflight (Table XVIII a). Thus, once the exposures had started, tortoises did not react immediately by holding still, but were likely to become still within a minute or so of exposure. The difference between the incidence of the behavior immediately before exposure was significant (Chi-square = 55.99, df=2, p < 0.0001). The bouts of stillness during exposures were interrupted by successive exposures, after which tortoises tended to look (half the incidences), retract their heads (11 of 12 incidences) or walk (11 of 17 incidences). This is why the duration of bouts was shorter.

In summary, the tortoises were observed to freeze (hold still) more frequently during the exposure period, but the behavior was interspersed with bouts of defensive head retractions, looking around, and walking. An examination of the total time spent in each activity showed that walk-

Table XVIII a-e: Summary of incidences of behaviors immediately before and after exposures to subsonic aircraft noise.

<b>A. Still</b>	<b>First Exposure</b>	<b>During Exposures</b>	<b>After Last Exposure</b>	<b>Totals</b>
Before stimulus	9	29	10	48
After stimulus	12	8	9	29
	21	37	19	77

<b>B. Head retraction</b>	<b>First Exposure</b>	<b>During Exposures</b>	<b>After Last Exposure</b>	<b>Totals</b>
Before stimulus	0	1	0	1
After stimulus	1	11	2	14
	1	12	2	15

<b>C. Look (still)</b>	<b>First Exposure</b>	<b>During Exposures</b>	<b>After Last Exposure</b>	<b>Totals</b>
Before stimulus	0	10	0	10
After stimulus	0	9	1	10
	0	19	1	20

<b>D. Walk</b>	<b>First Exposure</b>	<b>During Exposures</b>	<b>After Last Exposure</b>	<b>Totals</b>
Before stimulus	1	6	5	12
After stimulus	1	11	2	14
	2	17	7	26

<b>E. Digging and Climbing</b>	<b>First Exposure</b>	<b>During Exposures</b>	<b>After Last Exposure</b>	<b>Totals</b>
Before stimulus	5	6	2	13
After stimulus	2	5	3	10
	7	11	5	23

ing bouts were a particularly important component of the reaction. The total time spent holding still increased slightly during and after exposure (628 min before, 708 min during, and 773 min after). However, time spent walking (including investigating the barricade) increased greatly during exposures (173 min before vs. 337 min during vs. 161 min after).

The increase in walking suggested that tortoises became more active during overflights. However, time spent in energetic activities (climbing and digging) dropped by over half during and after exposures (digging: 220 min before, 93 min during, 51 min after; climbing: 170 min before, 13 min during, and 53 min after). Therefore, although the tortoises appeared to change their behaviors more often during exposures, they actually spent somewhat more time either still or in relatively less active states.

Long bouts of stillness (freezing) were uncommon. They were also confined to the first series of exposures of any individual. Habituation of this response occurred, in the sense that bouts of freezing in excess of 15 min were only seen during initial exposures. The time course of this habituation response was difficult to quantify because protracted freezing was rare.

#### Responses to simulated sonic booms

Responses to the sonic boom exposures were not as marked as those to subsonic aircraft overflights, and were often difficult to distinguish from normal activities altogether. The overall impression of observers was that tortoises sometimes looked around for the source of the impulses, but otherwise reacted little. The abrupt 'stop-motion freezing' was not observed and head retractions almost nonexistent (1 retraction during the entire period).

Because many of the exposures were well-separated in time (by hours), the during-exposure period was reduced. A total of 16.22 hr before exposure, 2.4 hr during, and 16.98 hr after were examined, yielding a total of 148 bouts of behaviors. Bouts of stillness, walking, and climbing predominated (Table XIX). No differences in the durations of these behaviors were found in the analysis (ANOVA,  $p > 0.05$ ). Total duration of bouts of stillness was slightly shorter after exposure (585 vs. 535 min) as was the total duration of bouts of walking (224 vs. 210), but the differences were small. Tortoises appeared to change their behavior more often in the hour after booms (3.83 bouts/hr vs. 4.18 bouts/hr), but the difference was too small to be significant.

Paired bouts of behaviors (immediately before vs. immediately after exposure) were examined (Table XX). These counts were taken from experiments exposing tortoises to single sonic booms

Table XIX. Incidences of the most common behaviors during exposures to simulated sonic booms.

Period	Before	During	After	Total
<b>Behavior</b>				
Still	20	1	29	50
Head retracted	0	0	1	1
Look	2	2	2	6
Walk	22	1	22	45
Climb	18	2	17	37
<b>Totals</b>	<b>62</b>	<b>6</b>	<b>71</b>	<b>139</b>
<b>Total Observation Time</b>	16.22	2.42	16.98	35.62
<b>Rate of Behavior</b>	3.82	2.48	4.18	3.90

Table XX. Incidences of behaviors immediately before and after exposure to simulated sonic booms.

Period	Before	After	Total
<b>Behavior</b>			
Still	10	11	21
Head retracted	0	0	0
Look	1	3	4
Walk	4	4	8
Climb	7	3	10
<b>Totals</b>	<b>22</b>	<b>21</b>	<b>43</b>

(Table IX, 10/16-10/18). Although sample sizes were small, there was no indication of change in behavior before and after exposure, let alone scaling with sonic boom peak overpressure (reactions were generally mild, so such scaling would have been difficult to detect without a large sample). Rates of climbing may have dropped after exposure, but the incidence of climbing was low and therefore difficult to interpret.

## Metabolic Rate and Heart Rate Responses

### Aerobic metabolism and heart rate

Resting metabolic rates ( $VO_2$ ) were measured for 8 tortoises (Figure 35).  $VO_2$  at rest ranged from 0.9137 to 1.996 ml/min/kg (mean = 1.472, s.d. = 0.410). Resting heart rates were also determined for 8 tortoises and ranged from 6.15 to 18.03 beats per minute (BPM; mean = 11.82, s.d. = 3.91; Figure 36).

In general, tortoises walked readily on the treadmill, though only at a limited range of speeds (Figure 37). When the treadmill moved too slowly or too fast, they dropped to the belt on their plastron, pulled in their legs, and allowed themselves to be carried to the rear of the belt, where they remained until rescued. Each tortoise had a clear preference for a particular walking speed, which it could maintain for periods in excess of an hour. Eventually, tortoises stopped measurement sessions by dropping to the belt and pulling in their legs. At that point, food and an avenue of egress, which ordinarily stimulated tortoises to walk, had little effect, suggesting that the tortoises eventually became tired. Verbal encouragement by animal care staff also proved useful for stimulating tortoises to walk; apparently, the tortoises had learned to associate

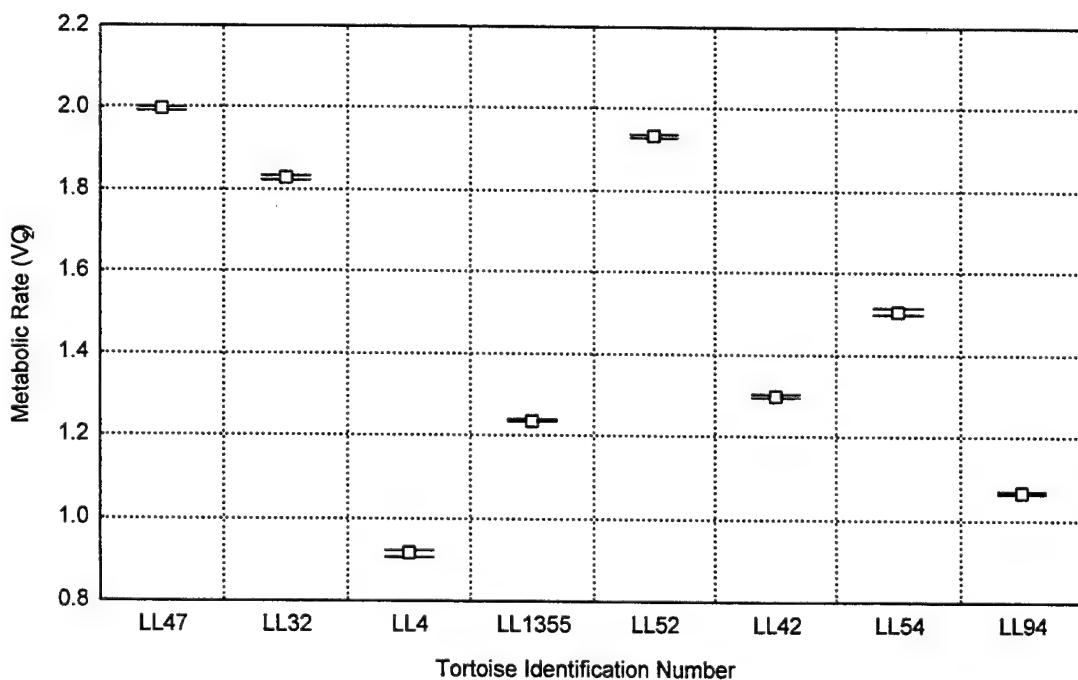


Figure 35. Weight-specific resting metabolic rate for 8 desert tortoises. Boxes indicate mean and bars indicate one standard error.

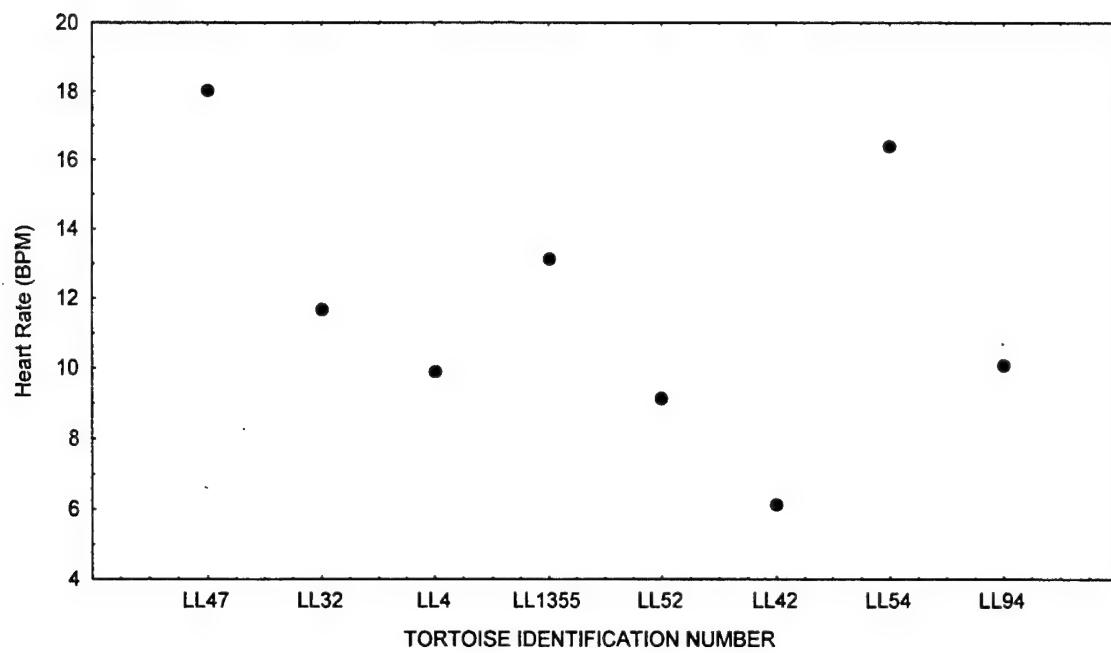


Figure 36. Resting heart rate in beats per minute (BPM) for 8 desert tortoises.

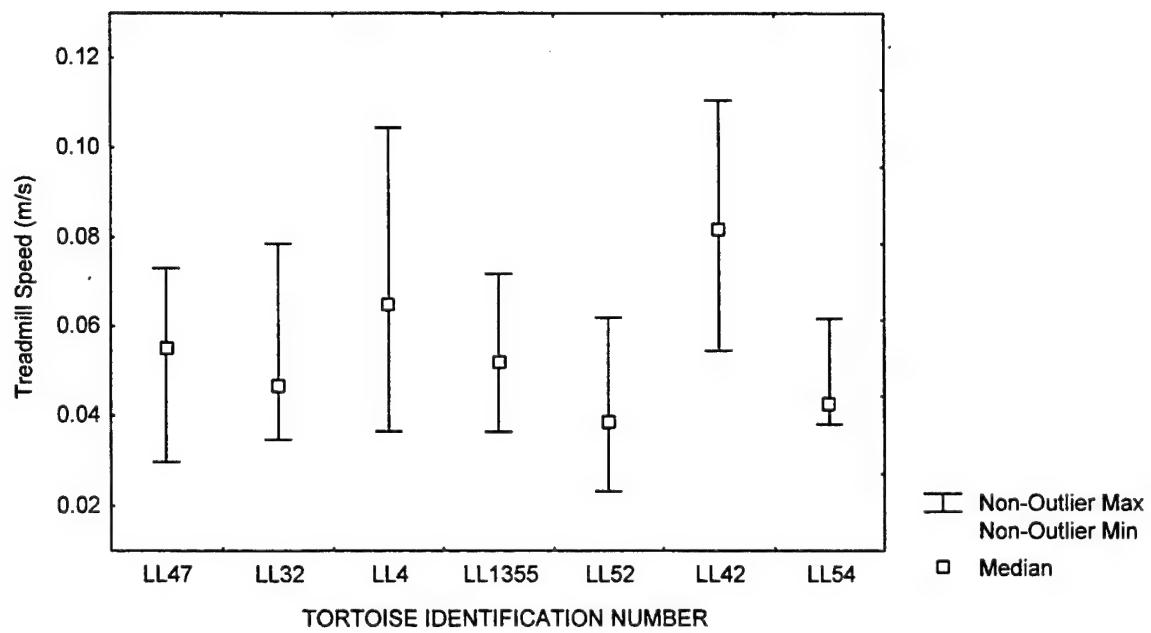


Figure 37. Range of walking speeds (m/s) of 7 tortoises walking on a treadmill.

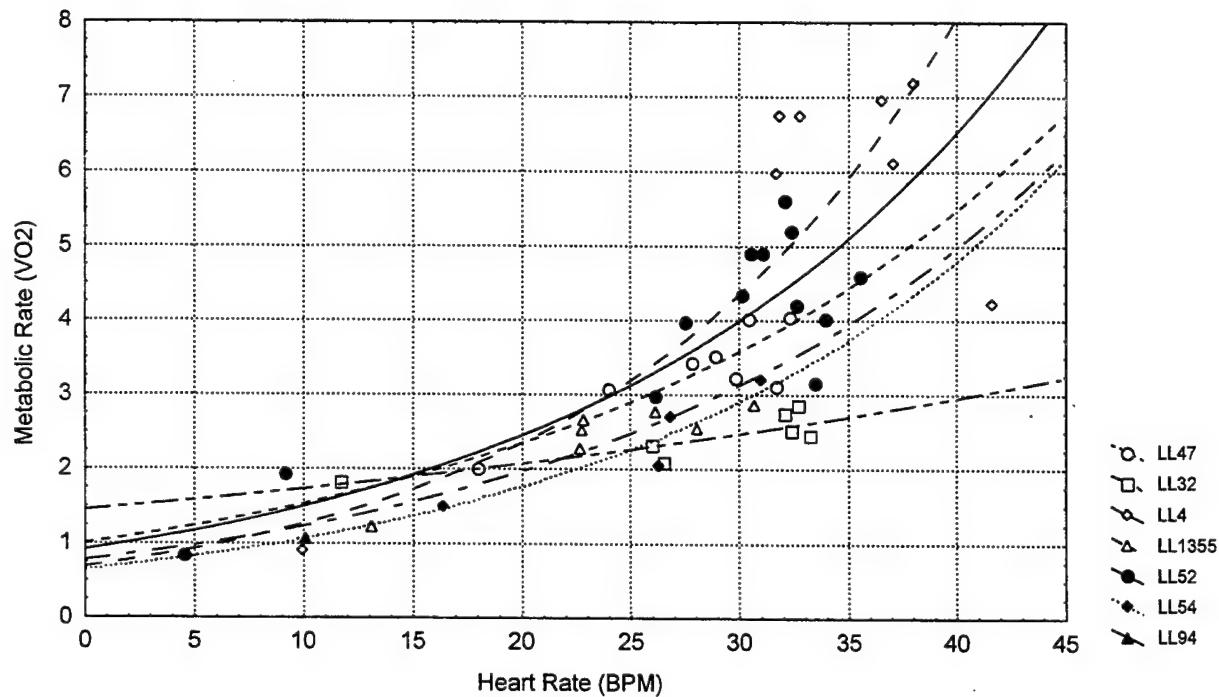


Figure 38. Correlation between metabolic rate and heart rate for 7 desert tortoises. Fit to an exponential curve for each individual is shown.

particular voices with food. Noxious stimuli (e.g., electric shock) were not used; the tortoises normally withdrew into their shells when exposed to noxious stimuli, the opposite of the effect desired. Table XXI lists the tortoises used in each of the three experimental protocols: walking with ECG monitoring only, walking with ECG and metabolic rate measurements, and walking with ECG, metabolic rate and serial blood sampling.

During walking, heart rate and metabolic rate clearly increased over resting rates (Figures 35 and 36 vs. 38). Maximal speeds in Figures 39 and 40 represent the maximum voluntary speed for each tortoise. Because the primary goal of these measurements was to obtain a predictive relationship between heart rate and metabolic rate for free-ranging tortoises, the tortoises were not forced to work outside their voluntary limits. However, the voluntary limit might not have been within the aerobic scope of the tortoises, particularly after walking for long periods. If the aerobic limits were exceeded, the measurement of oxygen consumption would underestimate true energy consumption, as it would not account for the anaerobic component of metabolism. Because a key indication of increased anaerobic metabolism is increased blood lactate levels, blood lactate was monitored for 5 tortoises at rest and during exercise.

Table XXI. Summary of tortoises used in measurements of resting metabolic rate, exercising metabolic rate, and exercising rate with blood sampling to determine blood lactate levels.

Tortoise	Resting	Exercise	Exercise with Blood Sampling	Resting Blood Lactate Concentration (mmol/l)
LL4	X	X <sup>1</sup>	X	0.5
LL 47	X	X	X	0.1
LL32	X	X	X	0.4
LL1355	X	X	X	0.5
LL52	X	X	X	0.4
LL94	X	X		
LL42	X	X <sup>2</sup>		
LL54	X	X		
LL93	X	X		
LL6	X	X	X	- <sup>4</sup>
LL1522	X	X	X	0.6 <sup>3</sup>
<b>Mean/SD</b>				<b>0.41±0.15</b>

Notes:

1 Resting value obtained after rather than before exercise experiment

2 O<sub>2</sub> analyzer apparently misread

3 Not fasted immediately prior to exercise experiment

4 Would not walk on treadmill

#### Blood lactate levels

The resting blood lactate levels are summarized in Table XXI for each tortoise monitored. At no time during any of the walking experiments was there a detectable increase in lactate levels in the blood stream. While the tortoises were encouraged to walk as fast as possible by increasing speed and extending the duration of the walk, tortoises ceased walking before reaching the aerobic maximum. Thus, the measurements of VO<sub>2</sub> represented the tortoises' true metabolic rate within the range of heart rates measured.

To determine the consequences of stimulating a tortoise to activity at which an increase in blood lactate was detectable, one tortoise (LL32) was held firmly while allowing it to attempt to free

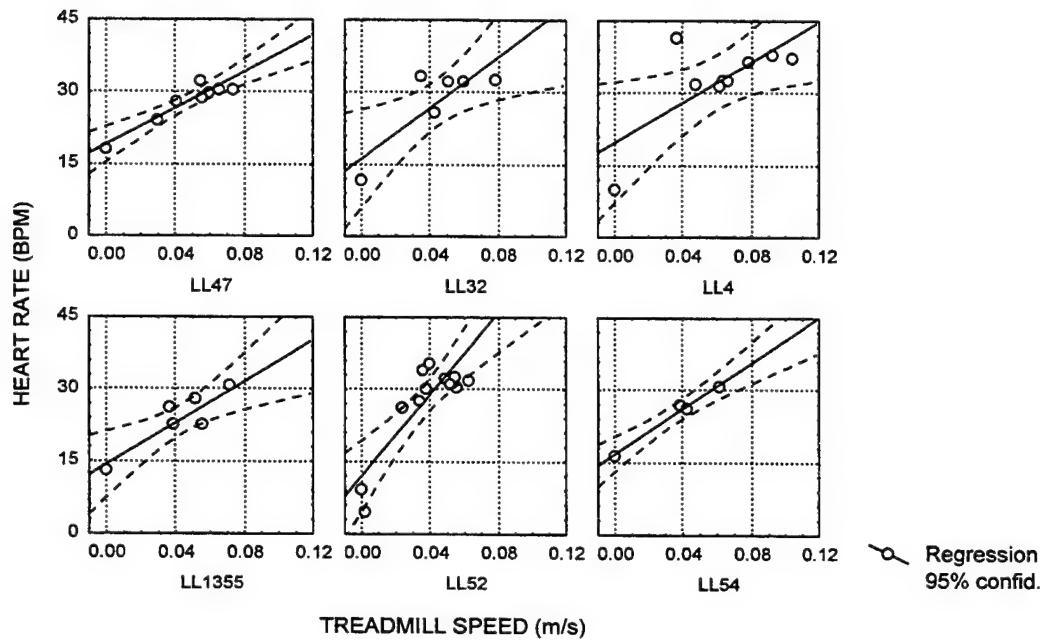


Figure 39. Relationship between heart rate and walking speed for 6 desert tortoises exercising on a treadmill. The solid line indicates the best-fit regression line and dotted lines indicate 95% confidence intervals. The identity of the tortoise is indicated under each curve.

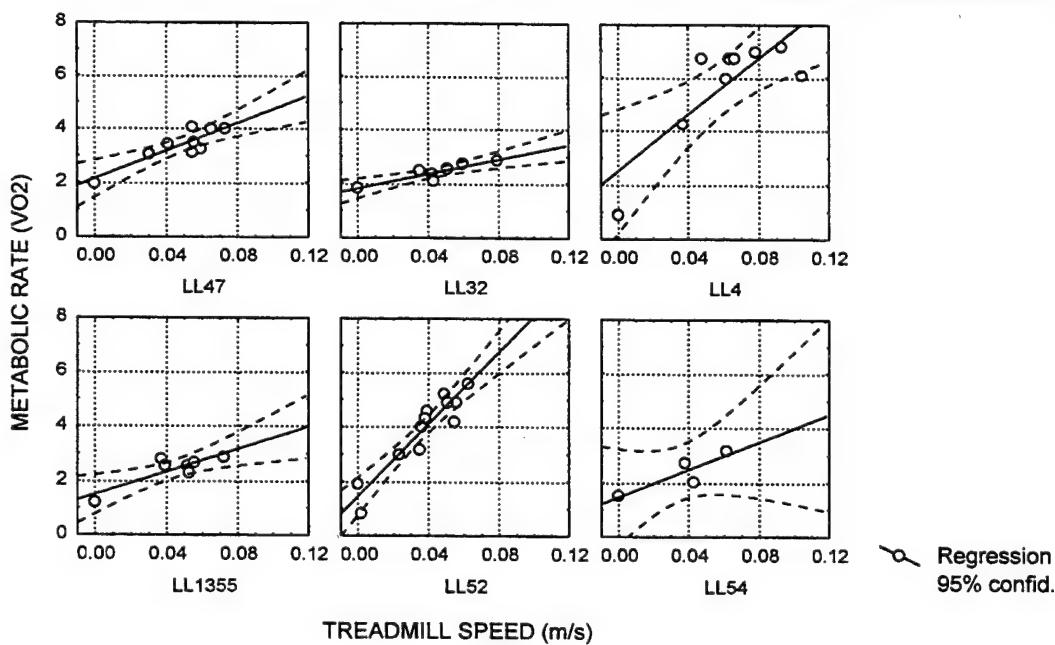


Figure 40. Relationship between metabolic rate and treadmill speed for 6 desert tortoises exercising on a treadmill. The solid line indicates the best fit regression line and dotted lines indicate 95% confidence intervals. The identity of the tortoise is indicated under each curve.

itself by pulling with its front legs against the holder's fingers. The tortoises worked hard to free themselves while being held as long as they could get purchase with their feet (falling upside down also stimulated persistent and energetic efforts of this sort). During a 45 min session during which this isometric exercise was stimulated continuously, heart rate and blood lactate levels were monitored. Blood lactate levels peaked at 2.7 mmol/l after approximately 22 minutes of sustained effort, with heart rate in excess of 41 BPM. Such sustained heart rate was far above what was normally encountered during voluntary treadmill exercise.

#### Relationship between heart rate and metabolic rate

Heart rate and metabolic rate were plotted against each other (Figure 38). The best fit to the data was an exponential curve. Because the data were relatively similar among tortoises and because it was desirable to obtain a model that could be used on wild animals without making metabolic rate measurements, the data were combined to establish a single model for the population (Figure 41). The correlation between heart rate and metabolic rate was excellent ( $r = 74\%$ ,  $r^2 = 55\%$ ). The residuals of the model were small and varied uniformly with increasing heart rate/metabolic rate (Figure 42).

#### Heart rate responses to simulated low-altitude jet overflights

In addition to the behavioral monitoring reported in the previous section, the study design called for measurements of heart rates and activity levels during exposures to simulated aircraft noise. Originally, the tortoises were to have been equipped with portable heart rate monitors for this phase of the study (Ultramarine Systems), which calculate and store heart rate per sampling period (usually about 4 s). However, the electrical signal detected from the electrodes in the carapace was never adequate to obtain an accurate estimate of heart rate using these units.

Measurements therefore had to be made from a continuous oscillographic trace with the polarity of the heart activity reversed. This required making measurements with the tortoises tethered to the heart rate monitoring system. Heart rates of seven tortoises were measured during exposures to subsonic aircraft noise (LL1522, LL1410, LL1, LL32, LL4, LL6, LL1355).

Tortoise heart rates were compared in the hour before, during, and after exposure to the 44 minute overflight tape for the first three days of measurement. There was no apparent trend in the three test periods (before, during, or after) or by exposure (1st, 2nd, or 3rd exposure of the day), or among the three tortoises (Figure 43-45, Table XXII). LL1355's heart rate declined during and after the 1st series of overflights, but her heart rate rose during the overflights on her 2nd and 3rd series. It declined in the hour after the second overflight, and increased in the hour after the third overflight series. LL1410's heart rate declined during the first overflight and

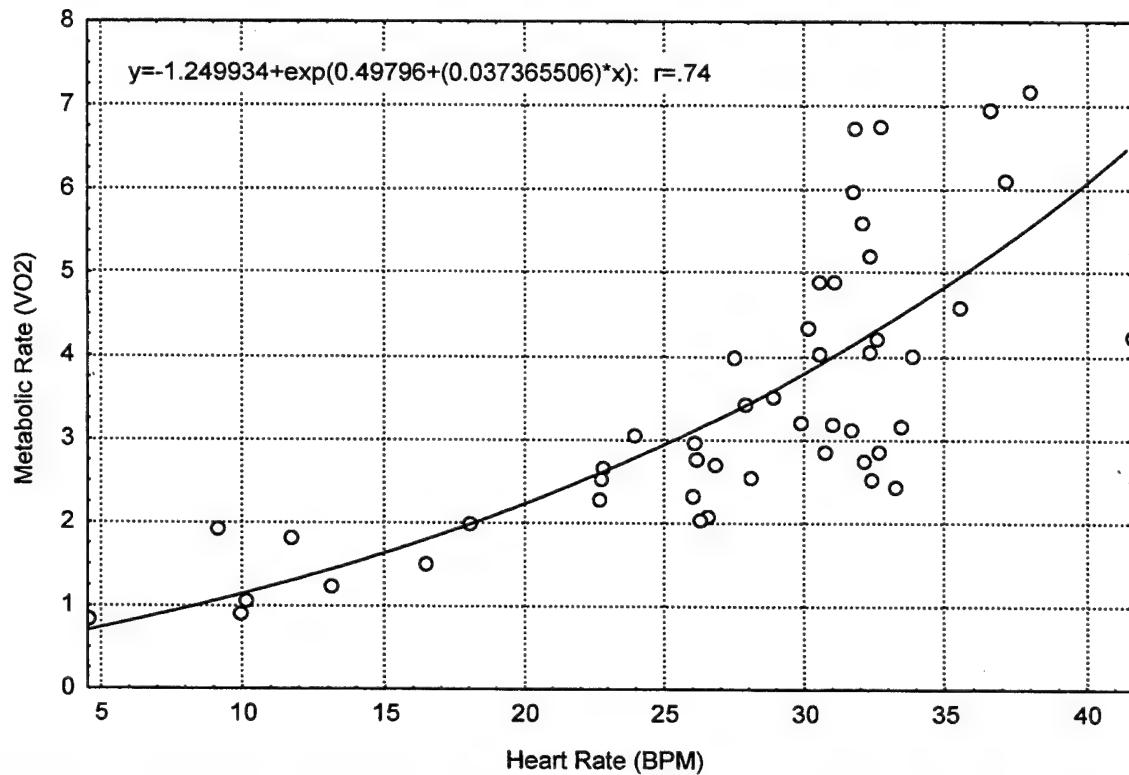


Figure 41. Regression of metabolic rate vs. heart rate (data for 7 desert tortoises combined). The best fit exponential model is shown.

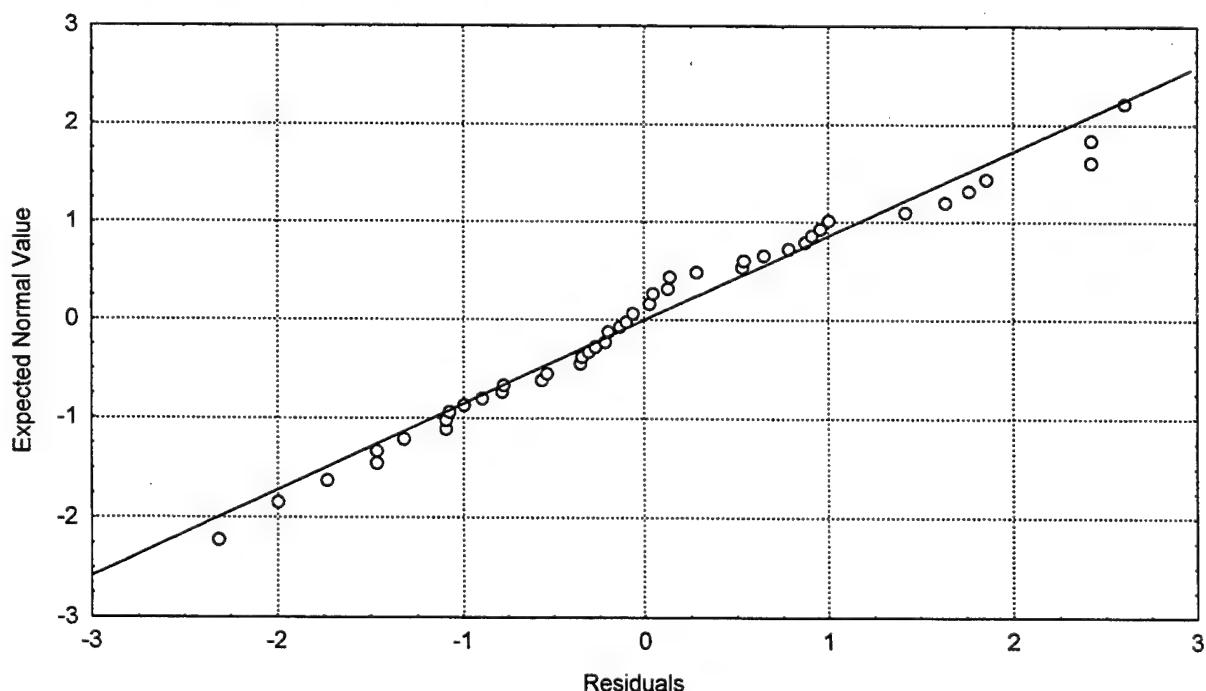


Figure 42. Normal probability plot of residuals for the exponential model of heart rate vs. metabolic rate.

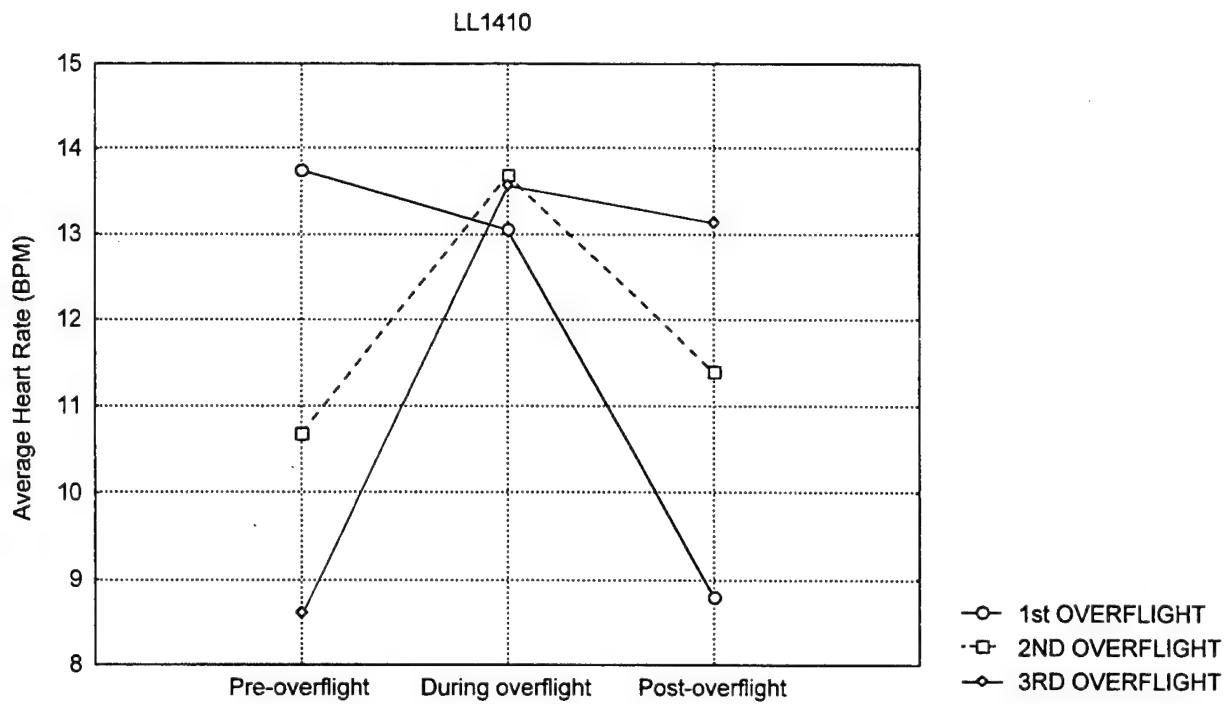


Figure 43. Heart rate responses to subsonic jet overflights by LL1410.

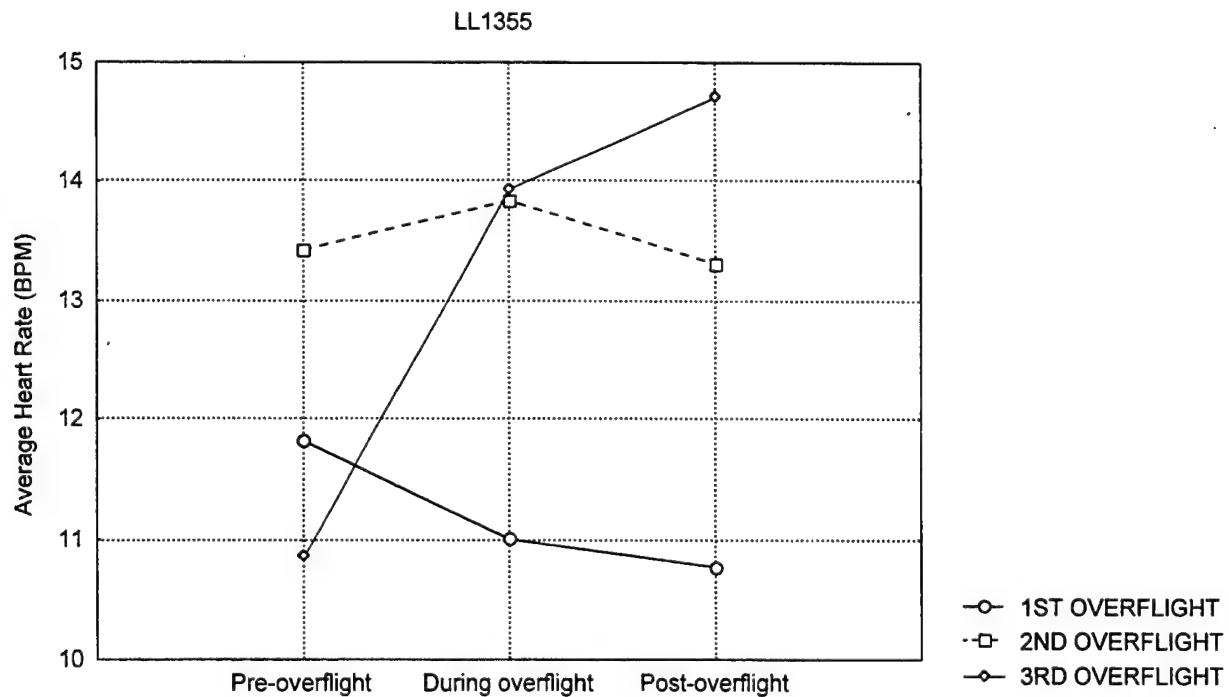


Figure 44. Heart rate responses to subsonic jet overflights by LL1355.

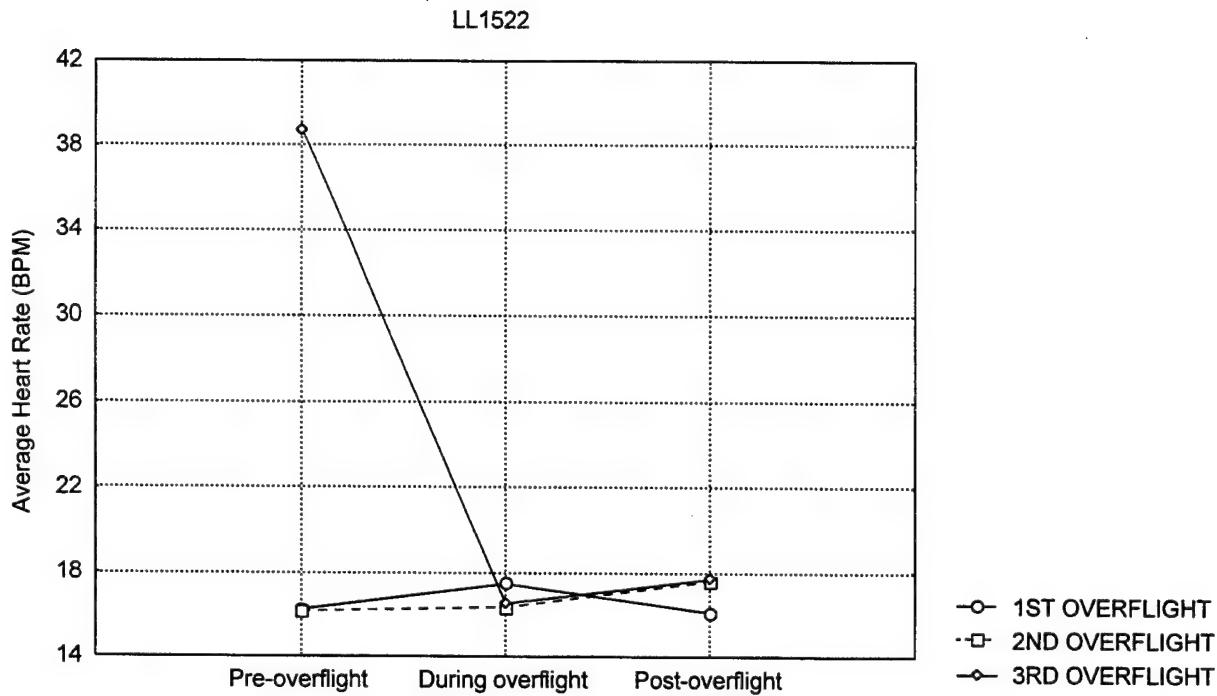


Figure 45. Heart rate responses to subsonic jet overflights by LL1522.

continued to decline an hour after. However, during the second and third overflight series, his heart rate increased dramatically and then declined to about 50% of the initial increase. LL1522's heart rate increased very slightly during the first flight series and declined back to her original level after the flight. Her response to the second overflight series was almost unchanged between pre and post overflights, with only a slight rise in heart rate post-overflight. On her third flight series her heart rate exhibited a dramatic decline (she stopped climbing) and remained low in the hour after the overflight series.

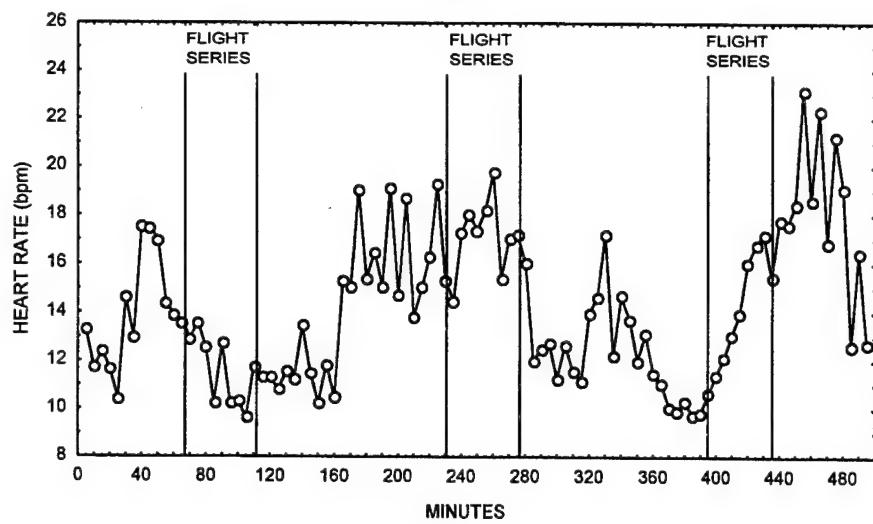
Heart rates were averaged in 5 minute blocks and plotted across the entire day for all the tortoises in these experiments (Figures 46-49). Heart rates increased when the tortoises were active (walking, climbing, digging) and decreased when they were still or looking. An examination of Figure 34 in comparison with Figure 46 (LL1) is instructive - the periods during which the tortoise's heart rate was high are also the periods when the tortoise engaged in short looking bouts interspersed with bouts of walking (during overflights) and climbing (especially after the end of the third overflight series).

As was the case for the behavioral analysis, a visual examination of heart rate before, during,

Table XXII. Mean, standard deviation, and t-test analysis of heart rate of desert tortoises before, during and after subsonic aircraft overflight exposures. Underlined p values less than 0.05.

		Mean Heart Rate								
Tortoise Number	Flight Series	Before Flight		During Flight		After Flight		Before/During	Before/After	After/During
		C	sd	C	sd	C	sd	p	p	p
<b>LL 1</b>	<u>1</u>	<u>13.95</u>	<u>2.37</u>	<u>11.75</u>	<u>1.62</u>	<u>11.74</u>	<u>1.39</u>	<b>0.028</b>	<b>0.011</b>	<u>0.979</u>
	<u>2</u>	<u>16.50</u>	<u>2.00</u>	<u>17.00</u>	<u>1.66</u>	<u>13.58</u>	<u>2.21</u>	<u>0.553</u>	<u>0.003</u>	<u>0.001</u>
	<u>3</u>	<u>11.52</u>	<u>1.67</u>	<u>14.08</u>	<u>2.41</u>	<u>18.09</u>	<u>3.28</u>	<b>0.010</b>	<u>0.000</u>	<u>0.006</u>
<b>LL 4</b>	<u>1</u>	<u>11.32</u>	<u>1.05</u>	<u>10.64</u>	<u>1.22</u>	<u>6.88</u>	<u>0.77</u>	<u>0.183</u>	<u>0.000</u>	<u>0.000</u>
	<u>2</u>	<u>7.68</u>	<u>0.72</u>	<u>11.62</u>	<u>6.52</u>	<u>15.24</u>	<u>8.10</u>	<b>0.050</b>	<u>0.004</u>	<u>0.286</u>
	<u>3</u>	<u>22.53</u>	<u>10.09</u>	<u>8.61</u>	<u>1.03</u>	<u>7.64</u>	<u>0.49</u>	<b>0.001</b>	<u>0.000</u>	<u>0.001</u>
<b>LL 6</b>	<u>1</u>	<u>12.68</u>	<u>2.25</u>	<u>10.41</u>	<u>0.504</u>	<u>9.64</u>	<u>0.54</u>	<b>0.008</b>	<u>0.003</u>	<u>0.011</u>
	<u>2</u>	-----	----	-----	-----	-----	-----	-----	-----	-----
	<u>3</u>	<u>12.73</u>	<u>3.23</u>	<u>8.62</u>	<u>0.732</u>	<u>9.49</u>	<u>0.80</u>	<b>0.001</b>	<u>0.003</u>	<u>0.019</u>
<b>LL 32</b>	<u>1</u>	<u>19.92</u>	<u>3.04</u>	<u>21.30</u>	<u>2.87</u>	<u>19.33</u>	<u>2.48</u>	<u>0.339</u>	<u>0.626</u>	<u>0.108</u>
	<u>2</u>	<u>19.33</u>	<u>2.48</u>	<u>15.26</u>	<u>1.94</u>	<u>16.11</u>	<u>2.65</u>	<b>0.001</b>	<u>0.006</u>	<u>0.424</u>
	<u>3</u>	<u>16.11</u>	<u>2.65</u>	<u>15.04</u>	<u>3.06</u>	<u>17.03</u>	<u>2.01</u>	<u>0.399</u>	<u>0.419</u>	<u>0.139</u>
<b>LL 1355</b>	<u>1</u>	<u>18.04</u>	<u>2.82</u>	<u>17.07</u>	<u>1.58</u>	<u>15.41</u>	<u>1.64</u>	<u>0.446</u>	<b>0.017</b>	<u>0.068</u>
	<u>2</u>	<u>15.41</u>	<u>1.64</u>	<u>14.47</u>	<u>1.61</u>	<u>15.01</u>	<u>1.29</u>	<u>0.223</u>	<u>0.526</u>	<u>0.403</u>
<b>LL 1410</b>	<u>1</u>	<u>14.82</u>	<u>2.67</u>	<u>12.69</u>	<u>1.21</u>	<u>10.38</u>	<u>2.95</u>	<b>0.039</b>	<b>0.001</b>	<b>0.040</b>
	<u>2</u>	<u>9.48</u>	<u>1.97</u>	<u>13.54</u>	<u>3.01</u>	<u>10.50</u>	<u>3.20</u>	<b>0.001</b>	<u>0.357</u>	<b>0.040</b>
	<u>3</u>	<u>8.77</u>	<u>1.18</u>	<u>14.70</u>	<u>4.59</u>	<u>16.09</u>	<u>4.37</u>	<b>0.000</b>	<u>0.000</u>	<u>0.497</u>
<b>LL 1522</b>	<u>1</u>	<u>22.14</u>	<u>2.72</u>	<u>22.00</u>	<u>2.02</u>	<u>21.23</u>	<u>4.08</u>	<u>0.895</u>	<u>0.524</u>	<u>0.610</u>
	<u>2</u>	<u>15.94</u>	<u>1.25</u>	<u>16.33</u>	<u>1.94</u>	<u>14.48</u>	<u>0.90</u>	<u>0.578</u>	<b>0.004</b>	<b>0.009</b>
	<u>3</u>	<u>19.98</u>	<u>3.02</u>	<u>19.19</u>	<u>3.06</u>	<u>18.35</u>	<u>2.71</u>	<u>0.561</u>	<u>0.177</u>	<u>0.513</u>

LL1



LL 4

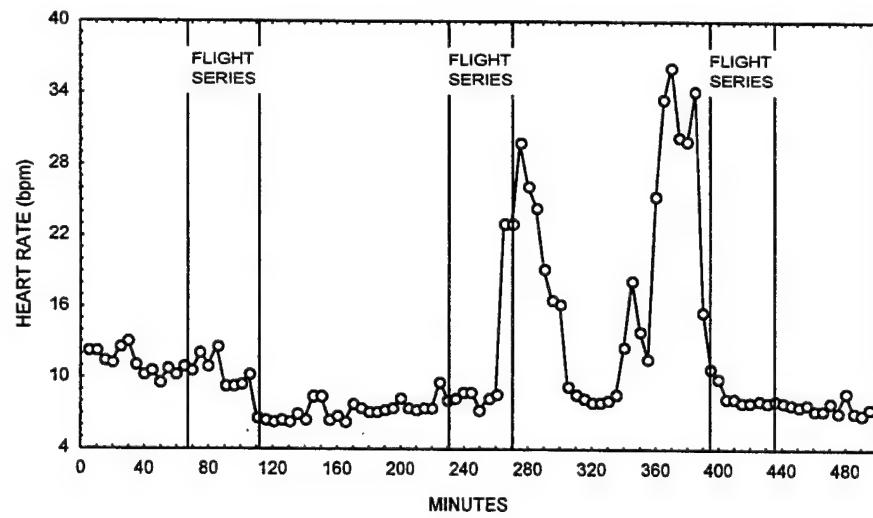
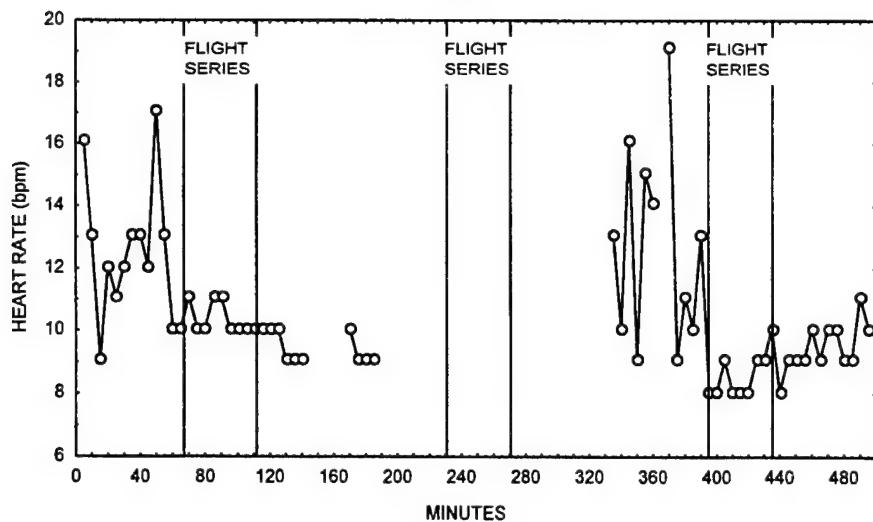


Figure 46. Heart rate of tortoises LL1 and LL4 before, during, and after exposure to subsonic aircraft noise.

**LL 6**



**LL 32**

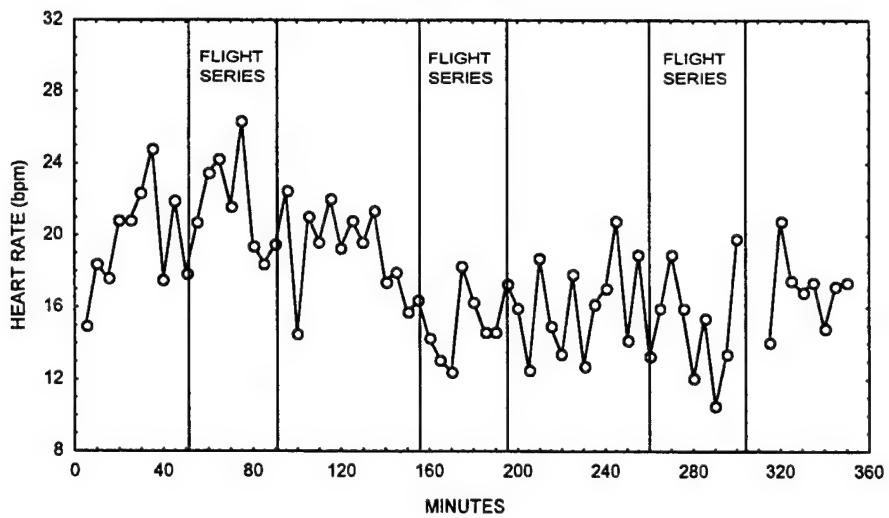


Figure 47. Heart rate of tortoises LL6 and LL32 before, during, and after exposure to subsonic aircraft noise.

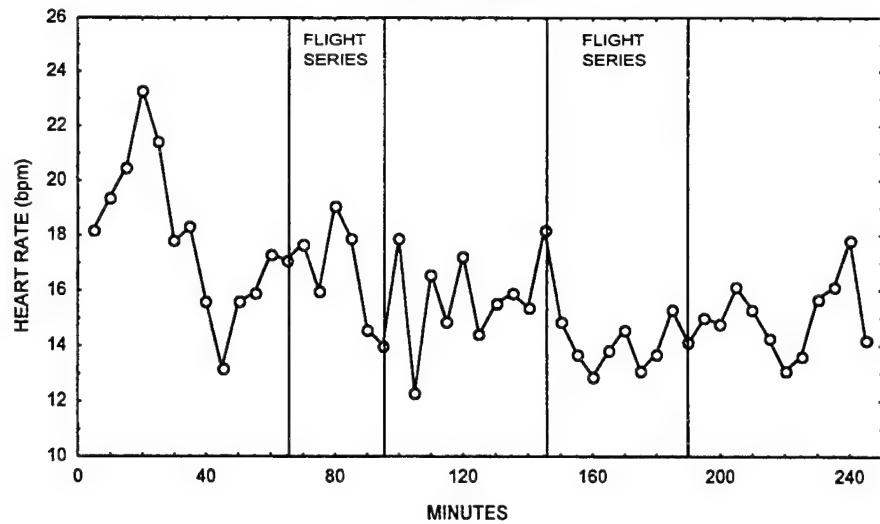
and after overflight series showed no obvious trends in heart rate (Figures 46-49), with some tortoises becoming more active during and after overflights, while other tortoises became less active. Samples consisting of the 5 minute averages for the hour block before, during, and after overflights were compared statistically (Table XXII). While it is unlikely that the 5 minute samples were completely independent of one another, tortoises changed their behavior every 2-5 min during exposures, resulting in corresponding changes in heart rate. The 12 samples collected in each period were compared statistically before *vs.* during, before *vs.* after, and after *vs.* during. Given the small sample, hence the low power, of each test, the criterion for overall significance was taken to be  $\alpha=0.1$ . However, conducting tests repeatedly was likely to produce falsely-significant results in a proportion of the tests. In order to eliminate the tests that were significant merely by chance, the criterion for significance of each individual test was taken to be 0.001 ( $0.001 \times 63 < 0.1$ ). Of the 63 tests performed, 6 could be expected to yield significant results by chance.

A total of 13 comparisons were significant by this criterion. Of those, 9 were significant decreases in activity during and after exposure. This result is consistent with the significant changes in behavior during and after exposure to subsonic aircraft noise (see previous section).

Figure 50 shows a Box-and-Whisker plot comparing heart rate before and after exposures for all tortoises. Heart rates were lower by 7.6% during the 60 minutes following the overflights. This is consistent with behavioral data suggesting that tortoises responded by 'freezing in place' and walking, rather than by continuing energetic climbing and digging.

Examination of successive interbeat intervals during the onset of overflights showed absolutely no sign of an increase or decrease resulting from the exposure. This supported the observer reports that the tortoises did not flinch, jerk, or breathe heavily after the onset of exposures. Any heart rate change that occurred were gradual or delayed by  $>15\text{min}$ .

**LL 1355**



**LL 1410**

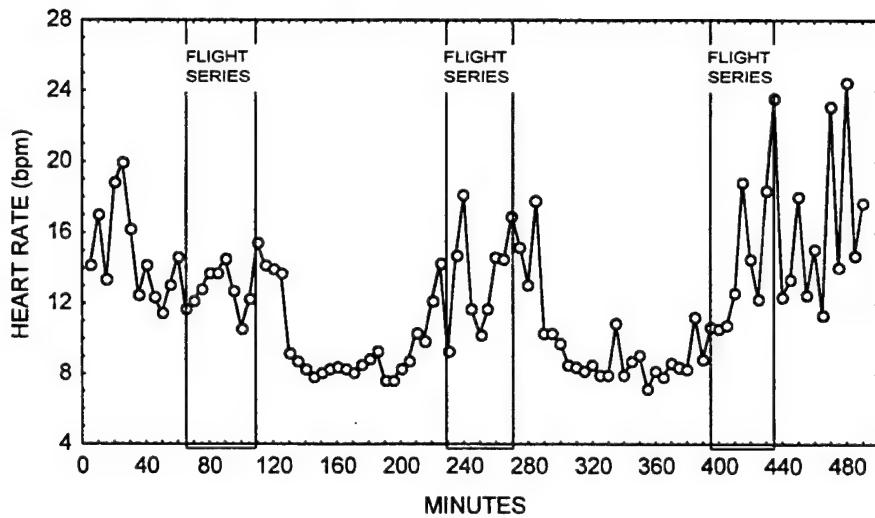


Figure 48. Heart rate of tortoises LL1355 and LL1410 before, during, and after exposure to subsonic aircraft noise.

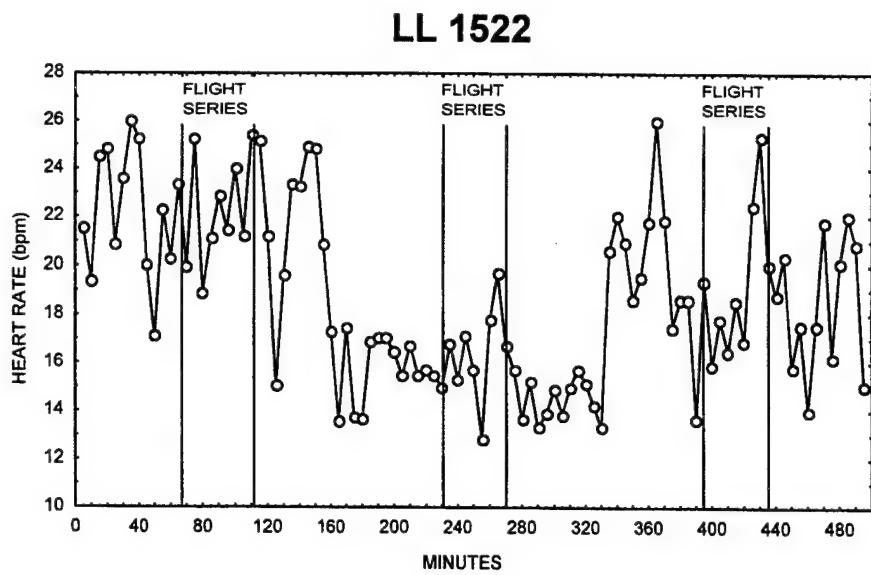


Figure 49. Heart rate of tortoise LL1522 before, during, and after exposure to subsonic aircraft noise.

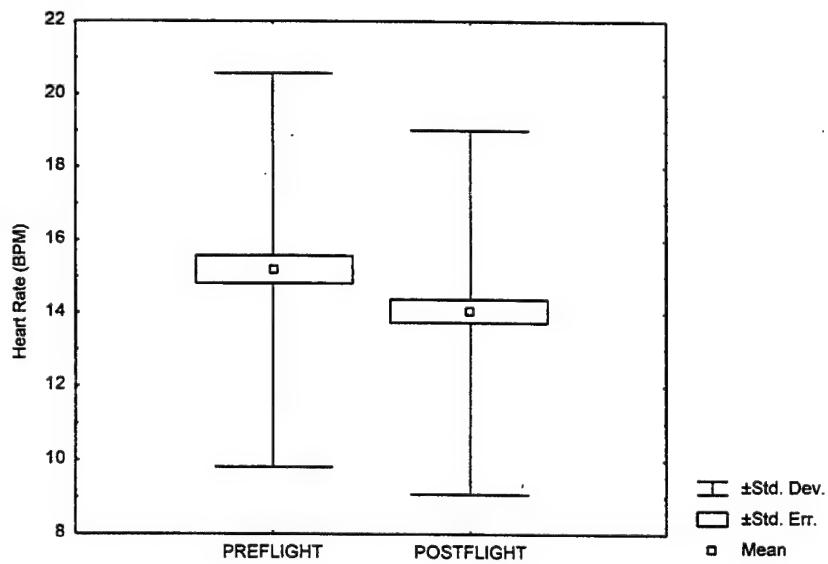


Figure 50. Box and Whisker plot of heart rate response to jet overflights.

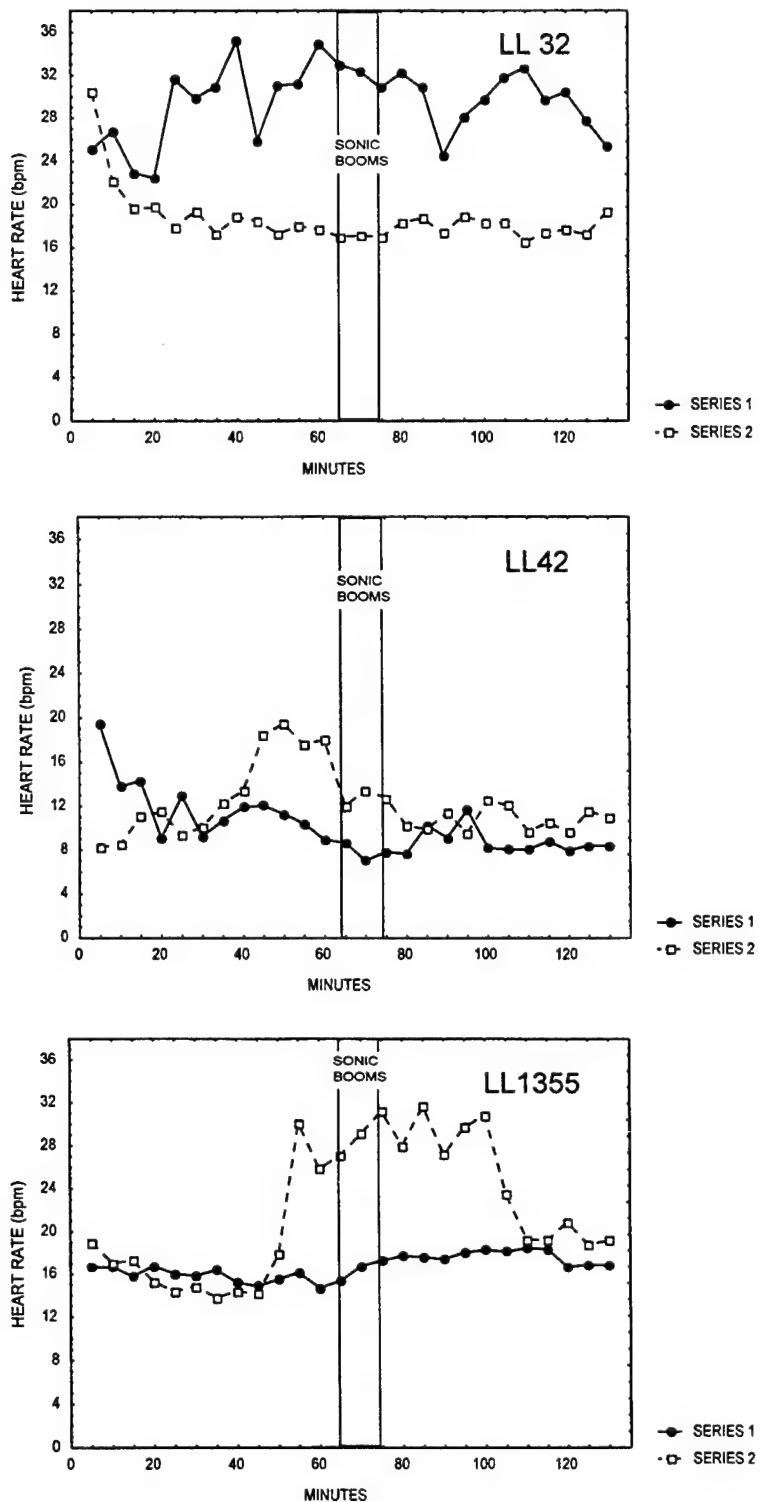


Figure 51. Heart rate of one desert tortoise before, during and after exposure to sonic booms delivered in blocks (Table V, IX). 5-min heart rate averages are plotted against minutes since the start of each of two experimental exposures.

### Heart rate responses to simulated sonic booms

Heart rate responses to simulated sonic booms were measured for 6 tortoises. Three of these were exposed to two blocks of booms (Figure 51) and three to four single booms over the course of a day (Figure 52). As with the overflight data, the 60 minutes prior to the sonic boom series was compared to the period during, and the hour after exposure.

Responses to the two blocks of booms (Figure 51) showed no consistent pattern among exposures or among tortoises. None of the large changes in heart rate that occurred during these observations could be linked in time with the onset of sonic boom exposures, either preceding them (LL1355, series 2) or following them (LL32, series 1).

Individual 5-minute averages were analyzed as for the subsonic aircraft noise data (Table XXIII). The same criterion for statistical significance was used in this analysis to facilitate comparisons between Tables XXII and XXIII. Four of 17 comparisons were significant (1-2 were expected by chance). Three of these were for decreases in heart rate and one for an increase. Average heart rate increased in 9 cases and decreased in 8. This analysis provided no evidence of a consistent change in heart rate during or after exposures. The pattern was similar for exposures to blocks of booms alone and for individual exposures.

A dependent 't' test analysis was conducted comparing heart rates in the 5-min sample immediately before and after exposure to individual sonic booms. This analysis demonstrated that there was no statistical difference between mean heart rate pre- and post- exposure when data from all tortoises were combined ( $p=0.15$ ). Box and whisker plots of the data from these two periods (Figure 53) make this clear. Not only was the variance large, but there was no obvious difference in mean heart rate between the two periods.

The heart rate data were entirely consistent with the behavioral data, consistent with the expectation that heart rate and activity should be closely linked. The combination of both types of data strongly suggested that sonic booms did not stimulate any significant changes in behavior other than brief bouts of looking.

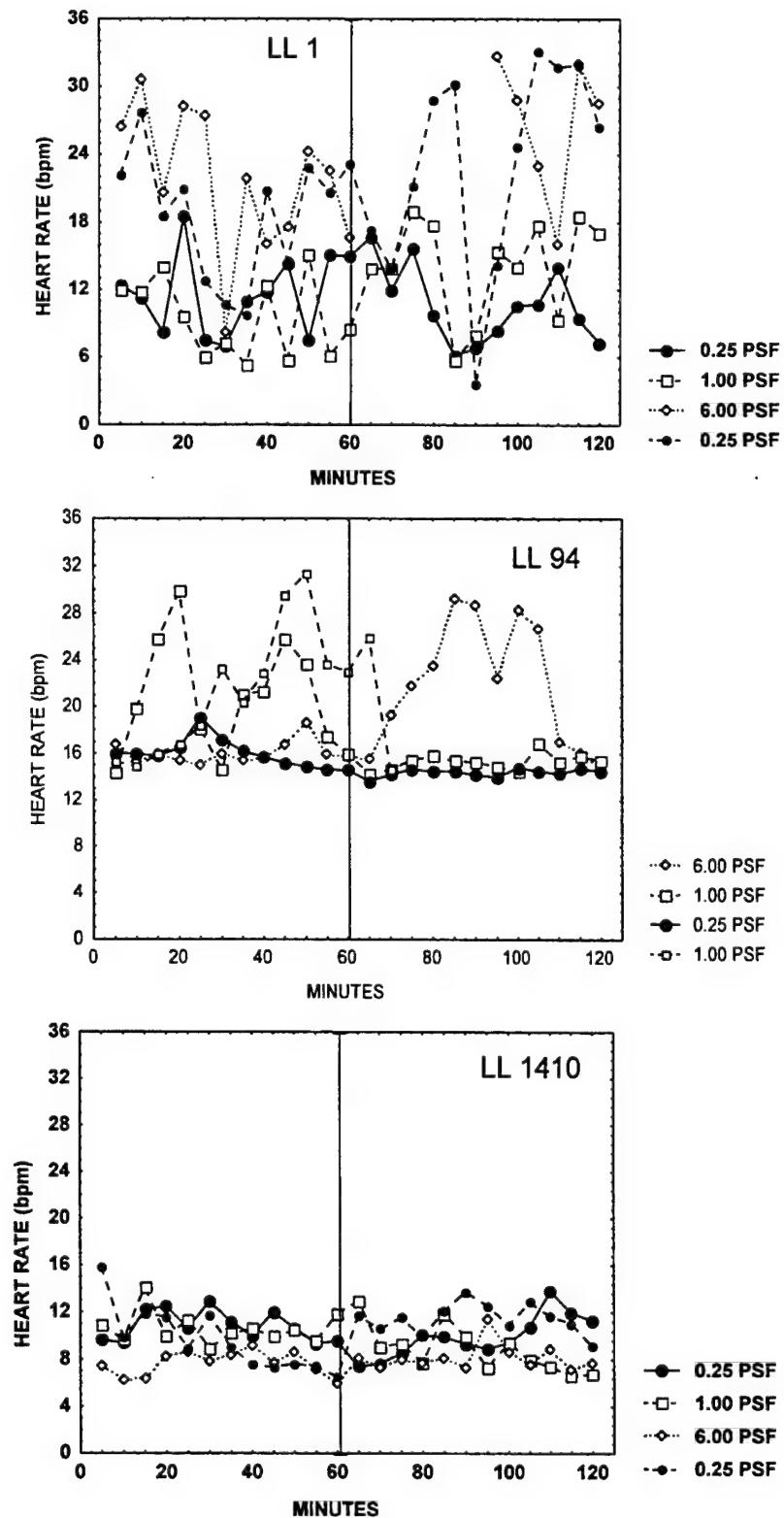


Figure 52. Heart rate responses of three desert tortoises exposed to individual sonic booms (Table IX). 5-min heart rate averages in the hour before and after exposure are plotted against minutes since the start of the observation.

Table XXIII. Mean, standard deviation, and t-test analysis of heart rate of desert tortoises before, during and after sonic booms.

		Mean Heart Rate				
Tortoise Number	Flight Series* or Level (psf)	Before Flight		After Flight		P
		C	sd	C	sd	
LL 1	0.25	11.28	3.67	10.91	3.49	0.804
	1.00	9.53	3.63	13.72	4.50	0.021
	6.00	22.17	6.42	25.35	6.93	0.334
	0.25	18.30	5.65	23.06	8.82	0.137
LL 32	SERIES 1	28.79	4.30	29.47	2.59	0.736
	SERIES 2	19.70	3.63	17.90	0.86	0.108
LL 42	SERIES 1	11.99	2.93	8.69	1.18	<b>0.001</b>
	SERIES 2	13.15	4.12	10.89	1.15	0.081
LL 94	6.00	16.06	0.99	21.46	5.47	0.004
	1.00	21.04	4.88	15.34	0.69	<b>0.000</b>
	0.25	16.08	1.22	14.38	0.33	<b>0.000</b>
	1.00	21.10	5.63	-----	-----	-----
LL 1355	SERIES 1	15.88	6.91	17.64	0.62	<b>0.000</b>
	SERIES 2	17.82	5.10	24.94	5.30	0.003
LL 1410	0.25	10.94	1.28	9.89	1.77	0.114
	1.00	10.50	1.35	9.09	2.06	0.065
	6.00	7.79	0.95	8.00	1.26	0.656
	0.25	9.88	2.65	11.09	1.84	0.202

\* For detailed list of booms in series, see Table V.

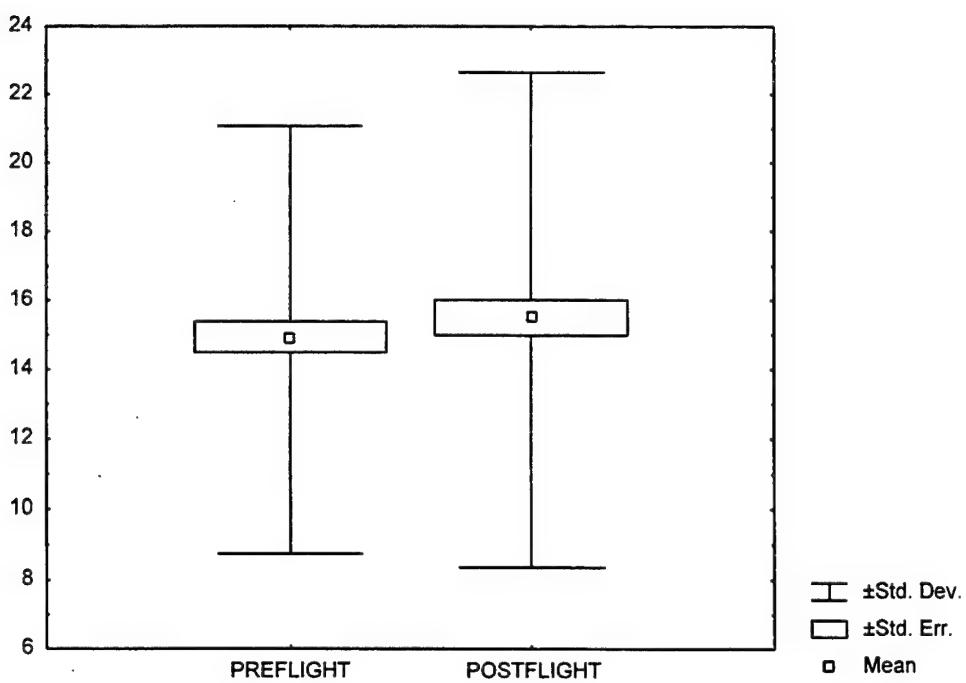


Figure 53 Box and Whisker plot of heart rate response to sonic booms.

## DISCUSSION

### Desert Tortoise Hearing

Desert tortoises proved to hear surprisingly well, based on the small amount of information on other species available at the start of the study (Patterson 1966, Wever 1978; Figure 2). Approximately half of the tortoises in this study had best sensitivities that were greater than expected, *i.e.* in the range from 20 to 40 dB SPL. Their good hearing was easy to understand in retrospect, as desert temperatures are high and high temperature increases the auditory sensitivity of reptiles (Wever 1978). The IAC chamber used in these experiments was kept within the temperature range from 28-32 °C, whereas the earlier behavioral studies of other species were conducted at room temperature (22-25 °C). Although this study did not target the relationship between temperature and sensitivity - few measurements were made at low temperatures - the data collected did suggest a substantial increase in sensitivity above 27 °C (Figure 30).

Conducting the experiments at summer temperatures may have increased the sensitivity of the tortoises, but another factor should have counteracted the increase - the difference in methodology between this study and the behavioral measurements of Patterson (1966). In this study, thresholds were estimated using far-field AEPs, which usually underestimate sensitivity by at least 5 dB, at least in the species normally tested by this technique (human babies, household pets). Unfortunately, for reptiles, the differences between thresholds collected using far-field evoked potentials and behavioral conditioning has not been quantified for any species. It may be that in these species, which are difficult to condition, the differences between behavioral conditioning methods and far-field electrophysiological methods are not as great. Therefore, while it is tempting to hypothesize that the present measurements underestimated tortoise sensitivity in the best range (200-700 Hz), there is no evidence to support such a contention.

At high frequencies (> 1000 Hz), the present measurements did not roll off as rapidly as expected based on Patterson's data. This flattening of the auditory threshold function is typical of electrophysiological measurements; therefore, desert tortoises are not likely to be more sensitive than other species in this range. The rolloff was as predicted at low frequencies (below 125 Hz; Figure 32).

When animals were collected for these experiments, individuals were chosen based on size (all adults) and general health - neither juveniles nor obviously unhealthy individuals were included. Other biases in the sample are possible as well. Examination of an unbiased sample of tortoises

will be needed to determine whether the proportion of sensitive individuals found in the test group is representative for the natural population. It would also be profitable to examine the effect of temperature on hearing in a more systematic way, as desert tortoises are active at temperatures ranging from 11 to 38 °C and are likely to receive sonic booms with little attenuation while in their burrows (Figure 17).

### **Effects of Aircraft Noise on Hearing**

Desert tortoises experienced small (5-20 dB) temporary threshold shifts after exposure to 10 sonic booms at high onset rate (0.4 ms) and short interval (3 s). Although 5 of 9 tortoises were affected, in all but one case, recovery from TTS was rapid - complete within the 45-60 min required to complete the post-exposure measurements. The individual with the greatest shift (20 dB) did not recover within the post-experimental period. Two days later, it was retested, and found to have a threshold 0 dB higher than during the baseline period at 250 and 500 Hz and 5 dB in response to the click stimulus (Table XV). Since the experimental error of the measurements was  $\pm 5$  dB, the animal was said to have recovered. The animal was not retested at shorter interval in order to avoid an overdose of sedative. These data suggest that the animal experienced a small to moderate shift, from which it recovered quickly. In humans and laboratory animals, shifts  $> 25$  dB (and usually  $> 40$  dB) are required to produce permanent damage. Further studies will be needed to determine the exposure level required to produce threshold shifts more directly usable as estimates of damage threshold - asymptotic threshold shift (the largest TTS that can be produced) or PTS (usually taken to be a shift  $> 15$  dB for more than 30 days).

The increased auditory sensitivity of the desert tortoise after exposure to simulated subsonic aircraft noise was unexpected and potentially significant. Several hypotheses were considered to explain the phenomenon: (1) experimental error, (2) a small increase in temperature during the post-exposure condition due to hysteresis in the regulating thermostat ( $+0.5$  °C), and (3) increased quiescence as the tortoise tired or became increasingly sedated over the course of the experiment. Experimental error was eliminated as a possibility by retesting animals on successive days; the error in measurements was found to be  $\pm 5$  dB, and as often positive as negative. Increase in temperature was unlikely to be a factor because thresholds at temperatures  $> 27$  °C were poorly-correlated with temperature and because the crucial measurement immediately after exposure was collected after the tortoise had cooled slightly in transit from the INTF.

Tortoise quiescence was most likely a factor because individuals were generally less mobile on the restraint board in the half hour after exposure. The best estimates of threshold were obtained when tortoises did not wave their legs or crane their necks on the restraint board during measurements. When the tortoise was active, muscle potentials made the AEP measurements difficult;

they were generally suspended until the tortoise quieted. Based on the results of measurements on the treadmill, tortoises did become tired after working for periods > 1 hr, while most of the playback experiments lasted for a little over two hours. Therefore, it is quite possible that in the last hour of the experiment, the tortoise was simply quieter, and that there was therefore less noise in the AEP measurements. However, fatigue does not entirely explain the behavior of the tortoises. The period of quiescence was always seen immediately after sonic boom exposures, and tortoises were normally as active during the last half hour of measurement as they had been at the start of the experiment. When no exposure was given (e.g., during measurements of hearing sensitivity, or retesting), the tortoises were as active in the second hour as in the first hour of the experiment. The quiescent period following an exposure was marked, lasting for 20 min to an hour. Other forms of behavioral suppression, for example tonic immobility, are accentuated by noise exposure (Gallup 1977), so the hypothesis that tortoises became unusually quiescent after exposure is not unreasonable. Whether this explains their greater sensitivity must be determined by further experiment.

#### **Behavioral Responses to Simulated Low-Altitude Aircraft Noise and Sonic Booms**

The tortoises with the best hearing were the most likely to approach at the sound of their keeper's voice, particularly LL54 and LL94. LL94 froze for the longest period (113 min) after exposure to subsonic aircraft noise. These observations suggest that sensitivity correlated with responsiveness to noise exposure in the desert tortoise. However, this correlation could not be established by quantitative measure. The reasons for this failure very clearly demonstrate the dangers of applying a perspective based on the mammalian acoustic startle to desert tortoises.

None of the desert tortoises in these experiments experienced an abrupt acoustic startle response of the sort common in birds and mammals, which is characterized by an abrupt increase in heart rate, loosening of the sphincters on bowels and bladder, vasoconstriction at the periphery, rapid mobilization of glucose reserves, muscular flinching, and other physical changes. The physical sensations associated with the startle are a major cause of aversion to loud noise in laboratory mammals (review in Bowles 1995).

The tortoises did not show any evidence of possessing a comparable acoustic startle response. They did not experience either abrupt increases in heart rate or muscular flinching, and did not defecate or urinate when exposed to either subsonic aircraft noise or simulated sonic booms. Such changes would have been detected had they been present - the tortoises jerked and fled when touched unexpectedly; they experienced an increase in heart rate at the same time; and they often urinated or defecated. They also jerked when a light was turned on or off abruptly. Apparently, they were capable of the vertebrate startle response, but were not stimulated to exhibit it by loud

transients.

Instead, the most acute response to noise was freezing, with slow changes in activity following thereafter. Freezing is a common reptilian defensive response (Suboski 1992) that seems very appropriate in the case of the desert tortoise, given its heavy armament. The physiological and behavioral processes that occur during an acoustic startle in birds and mammals are designed to potentiate the flight response (Davis 1981), a defensive behavior of little use to a tortoise. Its best strategy when it detects an unusual stimulus must be to cease motion, in the hope that any danger will pass it by. This is particularly true of loud sounds, which are often produced by sources at a great distance, *e.g.* lightning. Even in birds and mammals, freezing is a common response to loud sounds for this reason - better to become vigilant and see whether a sound materializes into an attack than to attract the attention of a predator by moving. For tortoises, freezing appeared to be the most intense of the possible responses to high-amplitude transients, and should be regarded as such by future studies.

In the most extreme case, the quiescent period resulting from freezing lasted 267 minutes out of a 16 hr day, or 28% of the day. However, this was an extreme response, and was confined to the first day of exposure. No bout of freezing lasted more than 5 minutes after the first day. More often, tortoises retracted their heads or looked around for the noise source. They shifted from energetic climbing and digging to less energetic holding still and looking, or walking and looking, or holding still for brief periods with the head retracted. This change in activity was confined to the period of exposure and the hour after (approximately), and was best characterized as a shift into an increased state of vigilance. Noisy or distracting activities such as climbing and digging were reduced at the same time.

In order to be detrimental, behavioral responses to aircraft noise would have to (1) significantly alter the 24 hr activity budget of tortoises under natural conditions, and/or (2) stimulate the tortoises to expose themselves to extremely unfavorable conditions (emergence in the heat of the day, activity in the presence of predators). The laboratory experiments reported herein did not find evidence for either potential effect. No detectable change in activity was observed after exposure to short-duration exposures (sonic booms), and even the strongest response (protracted freezing) diminished after only one day of experimental exposures, suggesting the potential for long-term habituation. When the present experiments were initiated, the evidence of Brattstrom and Bondello (1983) that spadefoot toads emerge from burrows more often in the presence of off-road vehicle noise led some commentators to suggest that desert tortoises might behave similarly. However, Brattstrom and Bondello did not demonstrate that spadefoot toads would emerge under adverse conditions in response to impulse noise, such as at noon on a hot day. In the laboratory,

tortoises gave no evidence of increased activity or reflexive behavior in the presence of sonic booms.

One final consequence of noise exposure on behavior was not measured during this experiments, but may be inferred from the results thereof - noise masking. The Desert Tortoise Recovery Plan (Desert Tortoise Recovery Team 1993) placed particular emphasis on this potential effect. Masking may be important under two conditions: (1) during an acute exposure, when an individual must respond appropriately to a danger signaled by sound, such as approach by a predator; and (2) during chronic exposure, when the individual may be prevented from hearing common signals such as social sounds. The fact that desert tortoises hear better than expected make such effects at least plausible, and they will be considered in turn.

Based on anecdotal accounts, desert tortoises respond to predator noise by orienting and looking at the source, just as they oriented and looked for the source of aircraft noise in the laboratory. During the period when an aircraft overflies a tortoise, masking of such sounds would be possible, given the high sound levels that may be produced by aircraft relative to soft sounds produced by a predator (e.g., it is perfectly conceivable that a 110 dB ASEL overflight would mask the soft rustle from a crow's wings). However, two factors will conspire to reduce the risk. First, tortoises are not known to depend greatly on their hearing to detect danger, except perhaps when they are very young. Second, the absolute level of aircraft noise on military training ranges is less important in the context of masking than duty cycle, the percent of time that high-amplitude noise is present. In fact, everywhere except in the immediate vicinity of a runway, the duty cycle of military aircraft noise is very low (2-3% of the time, or less; Bowles unpub. data). In most areas, any given location experiences fewer than 6 overflights per day. It would be a very unfortunate tortoise that was exposed to both a rare predator attack and a rare overflight at the same time.

The possibility of chronic interference with social signals is even more remote given such low duty cycles. If concerns over masking of social signals on the AFFTC ranges are to be realistic, areas must be found that receive much higher duty cycles than expected (> 10-20%) at times of day when tortoises are vocally-active.

#### **Effects of Aircraft Noise on Metabolic Rate and Heart Rate**

Desert tortoises proved to be excellent subjects for measuring exercise metabolism. They walked on a treadmill readily as long as the speed was not set too high or too low. Tortoises reacted to conditions outside their comfort limits with species-typical defensive responses, typically dropping the shell on the ground and retracting the head and legs. Optimal speed for the tortoises was

between 0.05 and 0.065 m/s. Often, they walked voluntarily at these speeds for more than an hour. As might be expected for a terrestrial vertebrate, heart rate and metabolic rate rose with exercise, with a good correlation between higher walking speeds and higher heart rates.

Resting heart rates varied with individual, ranging between about 6 and 18 BPM. In the data analyzed to date, there were no obvious differences in resting rate with sex or size of tortoise. Exercise heart rates ranged between 10 BPM and 42 BPM, with the most common rate during walking in the low 30's. It should be noted that none of these rates represent maximal output for desert tortoises; higher rates were observed when tortoises climbed on the walls of pens or burrows, and would probably have been seen when tortoises attempted to right themselves or during fights. Thus, the model of the relationship between heart rate and metabolic rate based on these results is limited to the activities that were observable during metabolic rate measurements. However, because these data represent voluntary behaviors by the tortoises, they are probably representative of most field conditions. The effect of temperature, torpor, and food deprivation on heart rate was not measured.

Metabolic rate correlated well with heart rate ( $r=0.74$ ;  $r^2=0.55$ ). Minimum resting metabolic rates were about  $0.9 \text{ ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$  ( $\text{VO}_2$ ), and maximum resting rates were about  $2 \text{ ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$ . One of the primary goals of this study was to determine if metabolic rate could be predicted from heart rate for the desert tortoise over a representative range of activity states, as is true of many other vertebrates. Figure 25 shows heart rate plotted against metabolic rate for 7 of the tortoises. Functions were fitted to these data for each individual tortoise, and, surprisingly, an exponential function fit the data best, rather than a linear function. The similarity among the tortoises was quite close, enough so that data could be combined to obtain a fit for the group as a whole. The resulting exponential function fitted the data very nicely, with a high correlation coefficient ( $r=0.74$ ) and small, unbiased fit of the residuals (Figures 41 and 42).

A possible explanation for the better fit of the data to an exponential rather than the more typical linear model might be the oxygen transport mechanism in this species. Three variables influence the rate of oxygen transfer by the circulatory system. These are heart beat frequency (= heart rate), cardiac stroke volume (volume of blood pumped per beat) and the relative quantity of oxygen withdrawn from arterial blood by the tissues (usually expressed as the arterial-venous ratio [=a-v ratio]). Barthomele and Tucker (1963) developed a formula to describe the relationship among these three variables and termed it the *oxygen pulse* or the amount of oxygen pumped by the heart in a single beat. Their formulation was later corrected by Gatten (1974). Most researchers quantify the oxygen pulse by measuring oxygen consumption and heart rate simultaneously. Oxygen pulse is then calculated by regressing the average oxygen consumption against

the average heart rate during a measured interval, expressed in milliliters of oxygen consumed per gram of body weight per beat.

Most studies assume that the relationship among stroke volume, the a-v ratio, and heart rate remains constant and linear across a variety of metabolic demands within a species (Barthomelew and Tucker 1963, Gleeson and Bennett 1985). However, Gatten (1974) found that for the slider turtle (*Pseudemys scripta*; at 10 °C) increased heart rate accounted for only 6% of increased oxygen transport, and for the western box turtle (*Terrapene ornata*) it accounted for 27%. For the desert tortoise, the results of this study indicate that, at higher metabolic rates, the rate of heart rate increase slows because other mechanisms are compensating. The most likely scenario is that the stroke volume increases during heavy exercise, thereby providing more oxygenated blood to the muscle at a relatively lower heart rate. Further research into the relationship of heart rate, stroke volume, the a-v ratio, and cardiac output in general is warranted.

The animals' aerobic limits also tend to make the heart rate-metabolic rate relationship non-linear. Eventually, as the tortoise reaches its maximum aerobic capacity,  $VO_2$  must reach its maximal limit. When that occurs the curve should 'top-over' or reach its asymptote. Thus, the final form of the curve should probably be sigmoidal and the best-fitting function should be a logistic function as opposed to a exponential function. At the upper limit, anaerobic metabolism becomes an important factor and heart rate no longer correlates well with energy consumed. For this study, tortoises were not exercised to  $VO_{2\max}$ , making it impossible to specify this upper limit.

There was a 7.6% decrease in heart rate after the overflight series for all tortoises. The results of our heart rate/metabolic rate correlation would predict that metabolic rate probably declined an equivalent amount. The observed reduction in activity of the tortoise post-overflight further supports this conclusion.

There was no discernable pattern in individual tortoise responses to sonic booms and for all tortoises there was no change in heart rates pre and post sonic boom. Thus, it can be concluded that sonic booms also did not impact metabolic rates of desert tortoises.

The effect of a small (7.6%) decrease in metabolic rate is difficult to infer without a better understanding of desert tortoise daily activity, daily energy budget, and habituation. A 7.6% reduction in metabolic rate for 1 hour cannot have serious consequences when viewed over a long period (a day, a week). Such changes are well within the normal range of variability of metabolic rate brought about by changes in diurnal activity. If one series of sorties actually distracted a tortoise from a critical activity such as foraging or digging, the animal's health and fitness would not be

compromised over the long-term. However, if overflights were repeated several times per day and the tortoise responded each time, significant effects would occur. Only experimentation on free ranging tortoises in heavily exposed areas can determine whether or not such effects can occur under real-world conditions.

### **Conclusions**

The present study did not yield any evidence that tortoise hearing could be compromised by carpet booms or low-altitude jet noise. Unusual exposure to focused sonic booms would be required to cause such damage, and the authors are not aware of any area where such unusual exposures could occur. The study also demonstrated that tortoises do not have an acoustic startle response that might stimulate them into panic flight or defensive urination. Because tortoises proved to be able to hear well enough to take advantage of soft sounds (*e.g.*, rustle of a predator's wings), it is possible that noise masking could result in occasional losses of young animals. However, the probability of masking effects on communication is vanishingly-low given the low duty cycle of aircraft activity in most military operations areas.

If research efforts are pursued in the future, several studies are recommended:

1. **Changes in activity should be investigated under natural conditions** in an area where exposures to aircraft noise is frequent. Activity should be measured under a variety of natural conditions, including extreme temperatures, hunger, water-deprivation, presence of predators, and presence of rivals.
2. **The model of metabolic costs developed here should be tested** with studies of free-ranging desert tortoises, which experience a wider range of conditions and activity states than the captives studied herein. How do desert tortoises regulate their energy and water budget in the face of natural challenges, such as heat and disappearing water sources? What role do endogenous rhythms and conditions play? What role do external stimuli play?
3. **The physiological events that potentiate freezing and quiescence in response to sound should be studied.** As it is clear that desert tortoises hear relatively well and are responsive to sounds in the environment, their sensory ecology should be examined. What are the meaningful natural sounds that they respond to? How well can they learn to recognize the acoustic signature of significant natural sounds and ignore irrelevant human-made noise?

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## APPENDIX A - GLOSSARY OF TERMS AND ABBREVIATIONS



## TERMS AND ABBREVIATIONS USED IN THE TEXT

Unless otherwise specified, all acoustical terms were drawn from Harris (1991).

<b>ABR</b>	<i>Auditory-evoked brainstem response</i> , an electrophysiological response evoked from the auditory brainstem using a sound stimulus.
<b>A/D</b>	<i>Analog-to-digital</i> converter.
<b>AEP</b>	<i>Auditory evoked response</i> . Any electrophysiological response evoked by a sound stimulus.
<b>AFFTC</b>	<i>Air Force Flight Test Center</i> , Edwards Air Force Base.
<b>ANSS</b>	<i>U.S. Air Force Aircraft Noise Simulation System</i>
<b>ASEL</b>	<i>A-weighted sound exposure level</i> ; a sound exposure level (SEL) is the time integral of squared instantaneous sound pressure over a given interval, referenced to 1 s. The sound pressure may be weighted (in this case A-weighted). Measured in pascal-squared seconds ( $\text{Pa}^2\cdot\text{s}$ ). In air, this quantity is referenced to 1 $\text{Pa}^2\cdot\text{s}$ (see definition of SEL below). An A-weighted SEL is obtained by filtering the signal using C-weighting prior to calculation of the SEL.
<b>BPM</b>	heart beats per minute
<b>CSEL</b>	<i>C-weighted sound exposure level</i> . A sound exposure level (SEL) is the time integral of squared instantaneous sound pressure over a given time interval, referenced to 1 s. The sound pressure may be weighted (in this case A-weighted). Measured in pascal-squared seconds ( $\text{Pa}^2\cdot\text{s}$ ). In air, this quantity is referenced to 1 $\text{Pa}^2\cdot\text{s}$ (see definition of SEL below). A C-weighted SEL is obtained by filtering the signal using C-weighting prior to calculation of the SEL.
<b>D/A</b>	<i>digital-to-analog</i> converter
<b>DAT</b>	<i>digital audio tape</i>
<b>EAFB</b>	<i>Edwards Air Force Base</i>
<b>ECG</b>	electrocardiogram
<b>EFR</b>	<i>envelope following response</i> , an auditory evoked response evoked by a steady-

	state, amplitude-modulated or frequency modulated sinusoid.
<b>Flat</b>	no weighting function applied to signal; equivalent to ‘unweighted’.
<b>fast</b>	<i>fast-weighting</i> , an integration time of 0.125 s.
<b>FFT</b>	<i>fast Fourier transform</i>
<b>FFR</b>	<i>frequency-following response</i> ; an electrophysiological signal that changes with the changing pressure of a sinusoidal input signal.
<b>ha</b>	<i>hectare</i> (metric abbreviation)
<b>HSWRI</b>	<i>Hubbs-Sea World Research Institute</i>
<b>Hz</b>	<i>Hertz</i> , cycles per second.
<b>IACUC</b>	<i>Institutional Animal Care and Use Committee</i>
<b>INTF</b>	<i>HSWRI Impulse Noise Test Facility</i>
<b>L<sub>max</sub></b>	Maximum, fast, A-weighted sound pressure level.
<b>ms</b>	<i>milliseconds</i> (metric abbreviation)
<b>overpressure</b>	The peak pressure of an impulse minus ambient pressure. Overpressure characterizes the degree to which an impulse exceeds standard atmospheric pressure at the point of measurement.
<b>Pa</b>	<i>Pascals</i> (metric abbreviation)
<b>peak</b>	The largest absolute value of sound pressure, usually measured in dB (peak sound pressure level). The peak occurs instantaneously, but must be measured over some period of time (sampling interval of a digital device, minimum response time of an analog device). Because some impulsive sounds achieve their peak within microseconds, this measurement can represent a considerable technical challenge. Normally, sound level meters are designed to provide some smoothing, using an exponential time weighting (e.g., fast integration time on a sound level meter is 125 ms).
<b>psf</b>	<i>per square foot</i> , the unit of pressure used to quantify sonic boom sound pressures. Correcting directly from this value to standard international units (Pa and $\mu$ Pa) is sometimes difficult because measurements were often collected as peak overpressures.
<b>PTS</b>	<i>Permanent threshold shift</i> , a decrease in auditory sensitivity that does not recover.
<b>s</b>	<i>seconds</i> (metric abbreviation).

<b>SEL</b>	<i>Sound exposure level.</i> Sound exposure is the time integral of squared instantaneous (usually weighted) sound pressure over a time interval equal to or greater than that of an event. The level is measured by the logarithm of the ratio of the time integral of squared instantaneous sound pressure over a given time interval. It is calculated as $SEL = 10 \cdot \log_{10} (E/E_r)$ , where $E$ is the sound exposure in pascal-squared seconds ( $\text{Pa}^2 \cdot \text{s}$ ) and $E_r$ is $20 \text{ mPa}^2 \cdot \text{s}$ (the product of the reference sound exposure [ $20 \mu\text{Pa}^2$ ] and reference time [ $1 \text{ s}$ ].)
<b>SNR</b>	<i>Signal-to-noise ratio</i> of a given sound, measured as the difference in dB between the sound level and the background noise level in the area.
<b>sound level</b>	unless otherwise specified, refers to fast, A-weighted sound pressure level.
<b>SPL</b>	<i>Sound pressure level.</i> In the context of hearing measurements made with tone pips or clicks, SPL is usually taken to mean RMS sound pressure level.
<b>STP</b>	<i>Standard temperature and pressure</i> , a standard condition used in studies of physiology, taken to be $25^\circ \text{ C}$ at 1 atmosphere.
<b>STPD</b>	<i>Standard temperature and pressure dry</i> ; standard temperature and pressure in a completely dry atmosphere.
<b>TTS</b>	<i>Temporary threshold shift</i> , a decrease in auditory sensitivity that recovers in a short time.
<b>URTD</b>	<i>upper respiratory tract disease</i> , any infection of the upper respiratory tract in desert tortoises. Suspected pathogens are given in Jacobsen <i>et al.</i> 1991.
<b>USAF</b>	<i>U.S. Air Force</i>
<b>VibBR</b>	<i>vibration-evoked brainstem response</i> , electrophysiological responses stimulated by a vibratory stimulus.
<b>VO<sub>2</sub></b>	metabolic rate (rate of oxygen consumption).



**APPENDIX B - ETHOGRAM (DEFINITIONS OF BEHAVIORS) USED  
IN ANALYZING VIDEO-TAPES OF PLAYBACK EXPERIMENTS  
WITH DESERT TORTOISES.**



## **Ethogram for the Desert Tortoise**

This ethogram is not an exhaustive list of the behaviors exhibited by desert tortoises, but is rather a list of behaviors that could be readily recognized on video during playback experiments and that were representative of the common activities of tortoises in the captive environment. A comprehensive ethogram for the desert tortoise is given in Ruby and Niblick (1994).

### **Events**

#### ***Defensive responses***

**Escape response:** Tortoise moves abruptly and directly into the burrow, wedges its shell in a corner, or runs across pen. In the captive environment, this response was only observed when humans approached a tortoise to handle it.

**Head withdrawal:** Tortoise pulls its head back, usually abruptly, but sometimes slowly; the head may be withdrawn all the way into the shell, but often is pulled back only partway; the legs may be pulled in simultaneously. In the captive environment, leg withdrawal was usual only when the tortoise was handled.

**Look :** Tortoise extends its head and looks around; it may stop other movements simultaneously (e.g., it may or may not stop walking). Looking is treated as an event only when it lasts <5 s. Tortoises most often interrupted a bout of stillness briefly to look or turned to look while walking.

**Shell drop:** The shell is dropped to the ground by retracting the legs partially or fully. Lying down was sometimes difficult to detect from the overhead camera in the sound isolation chamber, but was easily seen on the video from sonic boom experiments; the tortoise dropped its shell to the substrate, either as a preamble to rest or as a defensive movement (preparatory to pulling in the legs). It was not possible to distinguish defensiveness from the start of rest without other information (head withdrawal, abrupt looking).

**Still:** Tortoise stops all motion abruptly. Sometimes the head is retracted, but more often the tortoise simply ceases motion. If moving, the tortoise stops abruptly in mid stride; if climbing, the legs remain extended along the barricade; the frozen position is held for 5 sec or more.

This behavior was not referred to as “freezing” because, in practice, freezing was impossible to distinguish from any other bout of stillness unless the tortoise withdrew its head or froze in an awkward position (e.g., with one leg held up).

#### ***Social behaviors***

**Face rub:** Tortoise rubs its chin on its front legs; sometimes, the side of the head is rubbed on the legs as well; the portion of the head touching the legs was identified.

**Head bob:** Tortoise bobs its head up and down; this is similar to head-flagging in lizards.

### ***Comfort and maintenance behaviors***

**Mouthing:** When scored as an event, mouthing occurs when an object, including food, is handled briefly with the open mouth (< 5 sec); except for food, objects were mouthed only briefly.

**Urination:** Vegetable matter bedding showed urine spots clearly. Occasionally detectable in the video collected during experiments, but they usually discovered when the bedding is checked in the morning and evening. Assigned the time when it was first detected. Quantity was estimated by the amount of bedding affected.

**Defecation:** Occasionally detectable in the video collected during sonic boom experiments, but usually discovered when the bedding is checked in the morning and evening. Assigned the time defecation is first detected.

**Approach:** Tortoise approaches a keeper; typically the tortoises responded positively to a calling keeper by approaching with their necks stretched upward, presumably in search of food.

### **Activity States**

**Climbing:** Tortoise persistently attempts to climb the walls of the pen or the sides of its burrow, stopping occasionally to rest, look, or move a few paces. Often successfully reaches the top of the burrow, after which it usually stops for a time.

**Digging/burrowing:** Tortoise lodges itself in a corner or in the burrow, pushing bedding aside with its forelegs or ejecting bedding with its rear legs. When the burrow is not lodged against a wall of the pen, it may be shoved all over the pen in one of these bouts.

**Eating:** Tortoise grasps and chews food steadily (> 5 sec).

**Investigating barricade:** Tortoise walks along the margin of the barricade persistently, stopping to push its nose through the bedding, or climb the walls of the pen briefly. This behavior could also be called pacing.

**Invisible:** Tortoise out of view of the camera.

**Standing:** Tortoise pauses during other activities such as walking and either looks around, noses the bedding, or simply holds still, shell off the ground (> 5 s). Does not freeze—small movements of head and legs visible.

**Still:** Tortoise remains completely still with no observable head movements or leg movements. In this posture, the head, legs and shell are typically supported somehow, for example by lying on the ground or resting against a barrier or withdrawing into the shell. This posture may be held for > 1 hr.

**Turning over:** Tortoise rights itself after a fall; sometimes a very time-consuming process.

**Walking:** Tortoise walks around pen with occasional brief stops (< 5 sec), but is not trying to climb. While walking, tortoise may persistently nudge or mouth objects.

**Walking and looking:** Tortoise walks around pen; stops and looks or changes direction and looks (< 5) frequently. Bouts of walking with more than 3 looks were classed in this category.